Some aspects of the biology of the upstream migrating river lamprey, Lampetra fluviatilis (L.).

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SOME ASPECTS OF THE BIOLOGY OF THE UPSTREAM MIGRATING RIVER LAMPREY, *LAMPETRA FLUVIATILIS* (L.)

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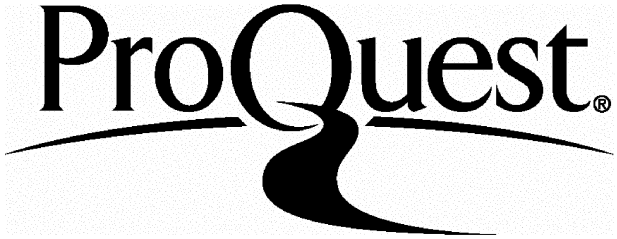

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SUMMARY

The biology of the early upstream migrant River lamprey has been studied using samples taken from the cooling water intake screens of the Oldbury Power Station in the Severn Estuary. Examination of the numbers of lampreys caught at different times suggests that increased fresh water discharge is the predominant environmental factor responsible for initiating the movement from the sea into the estuary.

The migrants could be separated into typical and praecox forms whose mean lengths were approximately 300 and 230 mm respectively. The typical forms were occasionally found in the estuary as early as July and as late as April with peak abundance generally being reached in November, whereas the praecox forms were present mainly between January and April.

The ratio of typical to praecox forms over the four years of sampling was approximately 4:1. In the typical forms, evidence was found for a correlation between high numbers and an increased proportion of males.

Measurement of a number of different characters, including gonadosomic and gut ratios, condition factors and body intervals, suggest that although the time when the typical forms enter the estuary is variable, the onset of the changes leading to sexual maturity are far more synchronous. A small number of the later typical migrants, however, exhibited different characteristics to those of the majority of the animals comprising this size category.

Measurements of the plasma constituents of animals from Oldbury indicate that they can regulate their ions in salinities at least as high as 70% of full strength sea water. The mean haematocrits and haemoglobin concentrations of approximately 40% and 14 g% respectively were greater than those of spawning animals. Differences were found between the ions of both the liver and the gonad of males and females. Muscle/plasma ratios for sodium and potassium were 0.056 and 33.23 respectively.

In the female River lamprey *(Lampetra fluviatilis)*, progesterone increased steadily from 31 ng 100 ml^{-1} in October/November to 1705 ng ml^{-1} in May. Testosterone levels in these females was very low in October/November (5.5 ng ml^{-1}) and undetectable thereafter. By contrast, in male River lampreys, testosterone concentration increased from 3331 ng ml^{-1} in October/November to 5344 ng ml^{-1} by May. Progesterone values in males showed a marked seasonal variation, ranging from 12.6 - 22.2 ng ml^{-1}.

In female *Petromyzon marinus*, captured in June at the onset of spawning, the testosterone concentrations ranged from 694 - 1669 ng ml^{-1}, with corresponding progesterone values between 1303 - 2367 ng 100 ml^{-1}. In males however, testosterone concentrations ranged from 2980 - 8846 ng 100 ml^{-1}, with progesterone values from 568 - 1241 ng 100 ml^{-1}.

1. INTRODUCTION

1.1. DISTRIBUTION AND LIFE CYCLES

Lampreys, together with the hagfishes, are the sole living representatives of the Agnatha, the most primitive group of Vertebrates. Their distribution is antitropical (Hubbs, 1952); a lack of ability to tolerate high temperatures apparently being the major factor in restricting their colonisation to the more temperate regions of the world (Lanzing, 1957; Potter and Beamish, 1975). Only three of the thirty-two species of lampreys are found in British waters (Hubbs and Potter, 1971). These are the Sea lamprey, *Petromyzon marinus* L., the River lamprey, *Lampetra fluviatilis* (L.) and the Brook lamprey, *Lampetra planeri* (Bloch). The first two species undergo an anadromous migration and are parasitic as adults while the third remains in fresh water and does not feed after the commencement of metamorphosis (Hubbs, 1925; Hubbs and Potter, 1971; Hardisty and Potter, 1971a, b, c).

The life cycle of all lampreys is characterised by a radical metamorphosis. The larval lamprey or ammocoete is relatively sedentary, living in burrows formed in the silt substrates of the slower flowing regions of streams and rivers (Hardisty and Potter, 1971a). It is blind and toothless and relies for food on the micro-organisms and detritus found in the water overlying the entrance to the burrow (Creaser and Hann, 1929; Schroll, 1959; Moore and Beamish, 1973; Moore and Potter, 1976a). After a number of years and a period of slow growth the animal transforms into an adult which is characterised by the possession of an oral disc on which there are

numerous teeth. Protruding from the central part of the disc is the piston which also bears teeth and can be used to rasp away tissues of host fish on which the lamprey has attached. Other characteristic external changes occurring during metamorphosis are the eruption of the eyes above the body surface and the enlargement of the dorsal and caudal fins. Internal changes are also radical and include the development of a new kidney (Youson, 1970), the conversion of the endostyle into the thyroid (Barrington and Sage, 1972) and marked changes in the pharyngeal apparatus which lead to the development of a tidal form of gill irrigation as opposed to the unidirectional method employed by the larvae (Randall, 1972; Lewis and Potter, 1976).

After the completion of metamorphosis, which takes several months, the adults of some species migrate to sea where they feed predominantly on host fishes (Hardisty and Potter, 1971b). Although information on the marine distribution of these anadromous species is limited, there is evidence that the larger species move further away from the coast than those which do not attain such a large size. This feature is illustrated by Zanandrea's (1962) comparison of the depths in the Mediterranean at which the large Sea lamprey was caught with those in the more coastal regions where the smaller River lamprey was taken. Furthermore, in North America the Sea lamprey has been collected from the Grand Banks far from its spawning sites in the rivers of Eastern Canada and the United States (Bigelow and Schroeder, 1948). The record of *L. tridentata* from Baja, California (Hubbs, 1967) and the Bering Sea (Svetovidova, 1948) and for *Geotria australis* from South Georgia (Ticknell, 1948; Permitin, 1966; Ivanova Berg, 1968) also support the view of a wide

ranging oceanic phase in species attaining the greatest size. These species also tend to have the widest overall distribution. For example, *P. marinus* is found in eastern North America and in north European and Mediterranean waters, whereas the smaller anadromous species, such as *L. fluviatilis* and *L. ayresii*, have a more restricted distribution (Hubbs and Potter, 1971). A similar generalisation applies to Southern Hemisphere lampreys. Thus, *Geotria australis* which attains lengths almost as long as *Petromyzon marinus*, is found in South America, Australia and New Zealand, while representatives of the smaller *Mordacia mordax* and *Mordacia lapicida* are restricted to south eastern Australia and the west coast of South America respectively (Potter and Strahan, 1968).

While some anadromous parasitic species have given rise to landlocked parasitic populations, of which the Sea lamprey in the Great and Finger Lakes of North America is a particularly good example (Smith, 1971), there are some parasitic species that are entirely restricted to fresh water (Hubbs and Potter, 1971). In general, such species are found in large river systems, e.g. *Ichthyomyzon* spp. in the Mississippi drainage basin (Hubbs and Trautman, 1937) and *Eudontomyzon danfordi* in the Danube (Zanandrea, 1951).

Some lampreys, however, do not feed at all after the initiation of transformation. In those species, the gonads develop rapidly during post-larval life and the animal reaches breeding condition some six to ten months after the first external metamorphic changes can be detected in the larvae (Hardisty and Potter, 1971c). It is now agreed by most workers that nonparasitic lampreys have been derived from either anadromous or freshwater parasitic species

(Hubbs, 1925; Zanandrea, 1959; Hardisty and Potter, 1971c). For this reason the distribution of each nonparasitic species can generally be correlated with its closely related ancestral parasitic form. Thus, the nonparasitic species of *Lampetra* occur in a considerable number of separate fluviatile systems, whereas those of *Ichthyomyzon*, *Eudontomyzon* and *Tetrapleurodon* tend to be found in a much more restricted number of rivers.

1.2 THE UPSTREAM MIGRATION

1.2.1. Timing of Migration and Spawning

Marked differences are found in the timing of the anadromous spawning migration in lampreys. For example, the Sea lamprey does not start entering the rivers until March or April with spawning taking place in late June or early July (Bigelow and Schroeder, 1948; Beamish and Potter, 1975). On the other hand, the River lamprey generally starts its migration in the autumn and upstream migrants have been caught even as early as the latter part of July (Berg, 1948; Lanzing, 1959; Hardisty and Potter, 1971b). Spawning takes place in the following spring between March and June, the latter breeding seasons tending to occur in the more northern rivers (Berg, 1948).

Although a migratory run commencing in the summer or autumn probably forms the predominant group in most populations of River lampreys, several workers have referred to a second group which starts migrating in the spring (Ivanova Berg, 1936; Berg, 1931, 1948).

Berg (1948) has suggested that the two groups be referred to as summer-autumn and spring forms respectively. In addition to distinguishing between these two groups on the basis of time of migration, Berg has also differentiated between the typical form of the adult River lamprey and that which he has termed the praecox form of this species. Thus, whereas the mean length of the typical form is in general approximately 30 cm, the praecox form is only 22 cm. Berg (1948) further differentiates between the two forms on the basis of the diameter and number of eggs. Several workers would not agree with Berg however that the River lamprey is composed of different groups, either on the basis of the time of migration or on the size at which they enter the rivers. For example, Gaygalas and Matskevichyus (1968) regard the movement from the Baltic into the rivers which they studied as occurring predominantly between September and November with little immigration from salt water taking place subsequently. After December, the animals in fresh water are not as active and only commence migrating again in the spring when they are approaching sexual maturity. The fact that the latter group were 13.7 - 18.8% smaller in length was attributed by Gaygalas and Matskevichyus (1968) to the shortening that is known to occur during the spawning run of lampreys.

The onset of migration in the landlocked Sea lamprey is initiated in the Spring at the time when water levels in the rivers rise above those of the lake (Applegate, 1950). Furthermore, the actual numbers moving at any one time during the migration is related to the temperature of the water. Both in the Sea lamprey and in the River lamprey, there are much data which suggest that movement follows a circadian pattern with maximum activity at night

(Enequist, 1937; Skidmore, 1959; Tesch, 1967; Claridge, Potter and Hughes, 1973). Moreover, catches appear to be greater on the darker nights, especially if these were found at the same time as higher water levels (Seligo, 1926; Buchholtz, 1938; Ryapolova, 1964). In view of these observations it is surprising therefore that Tesch (1967) should have discovered an apparent correlation between large catches in the River Elbe and times close to the presence of a full moon. Furthermore, he found no correlation between migratory activity and changes in either water levels or temperature.

The spawning of lampreys takes place in shallow areas where there is a gravel and sand substrate and where the water runs swiftly. Because of its size, the Sea lamprey is found in larger river systems and breeds in deeper water than either species of *Lampetra*. Moreover, in the very small rivers or streams only Brook lampreys are generally found. There are, thus, many cases when *L. planeri* is allopatric with its presumed ancestral species, *Lampetra fluviatilis*. A degree of ecological isolation through differences in spawning requirements may therefore have enhanced the processes of speciation of nonparasitic lampreys (Hardisty and Potter, 1971c).

Breeding in the Sea lamprey occurs between May and early July. The adults make a redd in the gravel using their oral disc to remove stones and thus form a depression. Approximately 200,000 eggs are buried in the sandy depression after fertilisation has taken place following simultaneous emission of sperm and ova (Hardisty and Potter, 1971b; Beamish and Potter, 1975). The River lamprey selects spawning sites where the water depth is not as great and the stones tend to be smaller. Although the mature eggs are of

similar mean diameter to those of the Sea lamprey (about 1 mm) the fecundity is much lower, lying in the range 7,500 - 28,000 (Hardisty, 1964). The nonparasitic *L. planeri* remains buried in the bottom sediments of the stream throughout the whole of its larval life and during most of the period of metamorphosis, finally emerging just prior to reaching full sexual maturity. The number of eggs produced are much lower than in *L. fluviatilis*, ranging from 1,000 - 3,000 (Hardisty, 1964). Again the smaller size of this species at maturity is reflected by a reduction in water depth and the size of substrate particles at the spawning site.

In all lampreys water temperature appears to be an important factor in the timing of spawning. For example, breeding in the Sea lamprey tends to be initiated at 15-16^{o}C (Applegate, 1950) while in the River and Brook lampreys the corresponding critical temperature is 10-11^{o}C (Hagelin and Steffner, 1958).

1.2.2. Changes In Body Morphology and Size

During the course of the spawning run, the adult River lampreys change from a bluish to a much more dull brownish colour. Another of the more striking external changes that occurs during the upstream migration is that the distance between the two dorsal fins becomes reduced until eventually the two fins are confluent (Hardisty and Potter, 1971b). This change may be correlated either with the growth of the fins and/or a shrinkage in the length of the body. Changes have also been observed in the relative size of the oral disc and the head region, a process that is so exaggerated in the Southern Hemisphere genera *Geotria* and *Mordacia*

that it has been referred to as a second metamorphosis (Dendy and Oliver, 1901). The increase in the size of the oral disc in the male of this species during the upstream migration also leads to a marked extension in the length of the preorbital region (Maskell, 1929; Potter, Lanzing and Strahan, 1968: Potter and Strahan, 1968).

By the attainment of sexual maturity the secondary sexual characters of the River lamprey are well developed. Thus, in the female the leading edge of the second dorsal fin becomes oedoematous and an anal fin develops just behind the cloaca whereas the males are characterised by the appearance of a urinogenital papilla (Hardisty and Potter, 1971b). These characters have been shown to be under the control of gonadal sex hormones (Evenett and Dodd, 1973).

During the course of the spawning run the animals also undergo a marked decrease in length and weight (Cotronei, 1924; Lanzing, 1959; Larsen, 1965, 1973). Ivanova Berg (1936) has reported shrinkages of 23% in males and 27% in females of the River lamprey with corresponding weight losses of 40 and 54%. Part of the overall reduction in weight is clearly attributable to the utilisation of the considerable stores of lipid accumulated at the end of the marine trophic phase (Moore and Potter, 1976b). The decline in length does not take place at the same rate throughout the migration. Larsen's (1973) data shows for example that for much of the early part of the upstream migration during the autumn and winter the loss is slight but becomes very marked during sexual maturation, especially in the female. Larsen considers that this reduction is due to mobilisation of body tissue and has shown that it can be reduced by hypophysectomy. There can be little doubt that the amount of length reduction found over a short period at spawning

is unique among vertebrates and reflects the poor development of the skeleton and the persistence of a notochord throughout adult life rather than the development of a vertebral column as occurs in other vertebrates (Larsen, 1962, 1973).

1.2.3 Changes In Internal Organs

Intestine

One of the most striking anatomical changes in the migrating lamprey is the gradual atrophy of the intestinal tract. Lanzing (1959) recorded a decline in gut weight from 1.0 to 0.1 g and a reduction in diameter from 7-10 mm to 1 mm. A similar pattern has been observed by Larsen (1973) who made the point that the rate of decline was most rapid in the earlier part of the spawning run.

The atrophy of the intestine is accompanied by degenerative changes in the epithelium and a shortening of the mucosal folds. Since the typhlosole does not undergo such marked degeneration as the other regions of the intestine, it eventually occupies virtually the whole of the lumen (Applegate, 1950; Hardisty and Potter, 1971; Larsen, 1973), and the foregut is said to be no longer patent at maturity (Weissenberg, 1926; Keibel, 1927).

That the degenerative changes are not irreversible has been shown by Larsen (1969, 1973) who found that after gonadectomy had been performed in January the mucosal folds increased in size and differentiated to such a degree that they took on the appearance of those found in animals collected in the autumn.

Liver

During the upstream migration of both River and Sea lampreys the liver undergoes a change in coloration (Applegate, 1950; Lanzing, 1959; Kott, 1970). Lanzing (1959) found that in the advanced stages of sexual maturity the reddish-orange liver turns to a light green in females and to a blue-green in males. This change in the colour of the liver is due to the accumulation of biliverdin (Sawyer and Roth, 1954) and bilirubin (Sterling, Wisten, Meranze and Krieger, 1967; Kott, 1970).

Lanzing's study (1959) on the hepatostomatic ratio of both the sexes shows an increase towards the end of the migrating period, a feature probably related to the marked decrease in body weight at this time. Later Larsen (1973) showed that there was an actual increase in males at spawning which she concludes must be due to an accumulation of protein and water. Several studies indicate that male River and Sea lampreys tend to have much higher liver fat content than females (Bentley and Follett, 1965; Kott, 1970; Potter and Moore, 1976b).

Gonads

During the migration the gonads steadily increase in volume and weight until they finally occupy the whole of the body cavity (Lanzing, 1959). In adult lampreys, the unpaired gonads are lobulate, elongated bodies, extending along the whole length of the abdominal cavity. There are, however, no special ducts for the evacuation of the genital products and in mature lampreys these products are shed freely into the body cavity. During spawning the sperm and ova enter

the urogenital sinus by means of a pair of abdominal pores before being released into the surrounding water.

The weight of the ovary, expressed as a percentage of the initial body weight, increases slowly during the autumn and winter, but rapidly from the end of February through to ovulation in the spring or early summer (Larsen, 1973). During the migration there is a gradual increase in the size of the eggs. This is accompanied by certain changes in the granulosa, the zone radiata and the yolk platelets (Lanzing, 1959; Larsen, 1973). Weissenberg (1927) found that whereas a female at the start of its migration possessed eggs with a diameter of 560 µm, the eggs of mature animals were 1173 µm. Ivanova-Berg (1933) stated that the length of eggs in River lampreys from the Baltic varies between 660 µm and 760 µm in October, increasing to about 1010 µm in May.

Apart from identification of oestradiol 17β and oestrone in ovarian extracts of the sea lamprey, *Petromyzon marinus* (Botticelli, Hisau and Roth, 1963) and some recent observations by Piavis, Dubin and Nardell (1975) on the oestradiol fraction in plasma extracts of the same species, there has been little published information on steroid metabolism in cyclostomes. Earlier attempts to demonstrate steroidogenic activity in ovarian tissue of the hagfish, *Myxine glutinosa*, were unsuccessful (Fernholm, 1972) but more recently, incubation of follicular tissue from another species, *Eptatretus burgeri*, with labelled precursors have shown that a number of the enzymes involved in steroid biosynthesis are in fact present in this group of cyclostomes (Hirose *et al*, 1975). Similar investigations have also been made on the interrenal and testicular tissues of parasitic phase Sea lampreys. After incubation with

labelled progesterone, the testicular tissue failed to produce testosterone, although 11-deoxycorticosterone was formed, indicating the presence of 21-hydroxylase activity (Weisbart and Youson, 1975).

The weight of the testis changes from October until the beginning of spermiation in March or April, the maximum weight being reached in January before decreasing through to spermiation (Ivanova-Berg, 1933; Lanzing, 1959; Larsen, 1973). At the onset of the spawning migration the testes contain spermatogonia with primary and secondary spermatocytes being produced during the autumn and winter.

The phase involving the change from spermatocytes to spermatozoa takes two months, all processes being relatively synchronous and taking longer than is normally the case in mammals (Larsen, 1973). Testosterone has been identified in extracts of the testis of *L. fluviatilis* and Δ^5-3β-hydroxysteroid dehydrogenases have been found in the somatic tissues of the male gonad (Hardisty and Barnes, 1968; Hardisty, 1971).

1.2.4. Osmotic and Ionic Composition of the Blood and Tissues

Although the hagfishes (Myxinoidea) and lampreys (Petromyzontida) are the sole survivors of the Agnatha, and are often placed together in the class Cyclostomata, these two groups differ markedly in a number of different aspects of their biology. One particularly good example is provided by the ionic composition and osmotic pressure of the blood. In the myxinoids, which are entirely restricted to marine environments, the blood is isosmotic or very slightly hyperosmotic to sea water (Robertson, 1957, 1974, 1976;

McFarland and Munz, 1958). This is due to the presence of high concentrations of inorganic ions in the blood plasma with sodium, and sometimes also chloride, being at a greater concentration than they are normally found in full strength sea water, whereas the reverse situation pertains with respect to magnesium, calcium and sulphate (Robertson, 1974; 1976). By contrast, both the freshwater and marine stages in the life cycle of those species of lampreys which undergo an anadromous migration have very much lower levels of ions in their blood (Morris, 1972; Robertson, 1974). This marked reduction in ions results in the osmotic pressure of the blood being equivalent to 1/4 or 1/3 full strength sea water.

These differences between the hagfishes and lampreys have been attributed to differences in the evolutionary history of the two groups (Lutz, 1975). Both the latter author and also Robertson (1957, 1974, 1976) have pointed out that the approximately isosmotic blood of the hagfishes parallels the situation exhibited by marine invertebrates and for this reason has therefore been taken as representing the primitive vertebrate condition. The hagfishes are therefore regarded as always having been a marine group whereas the lampreys, with their much lower blood ionic composition, would appear to have moved into fresh water early in their evolution or even to have actually evolved in fresh water (Lutz, 1975).

Although osmotic and ionic regulation in the *Petromyzonidae* has been fairly extensively studied, there are still gaps in our knowledge of the mechanism of marine osmoregulation due to the fact that only a relatively few live marine stages of any species of lamprey have been caught and analysed. The only available record for the osmotic pressure of the blood from a lamprey taken in seawater

is a freezing point value of -0.58°C (= 315 mosmoles) for a single specimen of *P. marinus* taken from the Mediterranean where the recorded freezing point depression of the environment was -2.3°C (Burian, 1910). This value for the blood is very similar to that reported by Fontaine (1930) for the stages of the same species caught early in their migration in freshwater. Although Bahr (1952) found that sexually immature *Lampetra fluviatilis* began to suffer in concentrations above 65% salt water, there can be little doubt that River lampreys are able to osmoregulate in fully marine environments. This view is based on studies in which downstream migrating stages of this species were shown to be capable of being acclimated, and even in many cases of direct transfer to full strength sea water (Potter and Huggins, 1973).

Upstream migrant River lampreys, which are able to osmoregulate successfully in 50% sea water, can maintain their blood plasma hypotonic to the environment and increase their water content by as much as 30% in spite of osmotic losses of water (Morris, 1958). Morris (1958, 1960) and Pickering and Morris (1970) have shown that they use a mechanism which is remarkably similar to that employed by marine teleosts (Potts and Parry, 1964). It involves the drinking of sea water, which means absorbing a solution of ions, the excess monovalent ions then being excreted by means of specialised ('chloride excreting') cells located in the gill epithelium. Most divalent ions are left in the residual fluid of the gut while those which enter the body fluids are excreted (Pickering and Morris, 1970).

Studies on the mechanism of osmotic regulation of anadromous lampreys show that the plasma osmotic pressure declines during the spawning migration, with the osmolarity falling very markedly during

the spawning period, finally reaching levels comparable to that of the ammocoete. During spawning, the osmolarity of the sera of landlocked Sea lampreys has been given as 257 mosmoles (Mathers and Beamish, 1973), a value very similar to the 266 mosmoles found in anadromous spawning *P. marinus* by Pickering and Morris (1970). The decrease in both the osmolarity and the ability to osmoregulate in *L. fluviatilis* at spawning may be caused by a number of factors including increases in skin permeability (Hardisty, 1956; Morris, 1958), degeneration of the kidney (Youson, 1970), loss of the 'chloride output cells' and a breakdown in the swallowing mechanism (Pickering and Morris, 1970).

The ionic constituents of the blood of ammocoetes and of adult lampreys after they have re-entered fresh water have been investigated in some detail by a number of workers (Fontaine, 1930a; Wikgren, 1953; Hardisty, 1956; Morris, 1956, 1958, 1971; Bull and Morris, 1967). Their results show that there is a very efficient homeostatic mechanism for controlling ions and water balance at both stages of the life history. In its ionic composition the ammocoete larva resembles that of many freshwater vertebrates (Potts and Parry, 1964).

Studies carried out at various stages of the life cycle of the landlocked Sea lamprey show that virtually all the ions are higher in immature adults than in the larval stage, despite the fact that this animal remains in freshwater for the whole of its life cycle (Urist, 1963; Urist and Van der Putte, 1967). With respect to *L. fluviatilis* there is considerable disagreement over the levels of chloride in the blood, a divergence being found in both the larva and adult. For example, in the ammocoete the plasma chloride is

given as 93.8 mM l^{-1} by Bull and Morris (1967) and as 58 mM l^{-1} by Hardisty (1956). Likewise, in immature adults, values between 92 and 95.9 mM l^{-1} are given by three different workers (Galloway, 1933; Robertson, 1954; Pickering and Morris, 1970), whereas Hardisty (1956) reported a level of 113 mM l^{-1}.

Ionic Composition of Tissues

As has been pointed out by Robertson (1974), very little is known about the ionic composition of lamprey tissue, apart from reports of analyses of ions in the whole animal. Measurements of chloride in adult *L. fluviatilis*, collected in November, showed 43 mg-ions kg body water^{-1} and in March after the period of fasting and sexual maturation 55-ions kg body water^{-1} (Hardisty, 1956). *L. planeri* ammocoetes collected in June had smaller total chloride contents (25.2 mg-ions kg body water^{-1}) than the adults. The mean sodium content of *L. fluviatilis* adults living in tap water has been given as 38 and potassium as 71 $\pm$ 1.1 mg-ions kg body weight^{-1} (Bentley and Follett, 1963). Recently Nesterov (1972) has studied the intestine, heart, body wall and tongue retractor muscle of *L. fluviatilis*. He found that the gut wall was of tonic muscle, resembling that of molluscs, with which it had many common features, such as a similarity in the concentration of Na^+ and K^+.

1.2.5. Haematocrit and Haemoglobin Concentration

Another striking feature of the spawning run of lampreys is the decline that takes place in the number of red blood cells following the entry into freshwater (Ivanova Berg and Sokolova, 1959).

Thus, in the autumn, the number of erythrocytes in 1 mm^3 of blood was 1065 x 10^3, which corresponds to an haematocrit of 36.5, whereas in the following spring the respective values were 1014 x 10^3 and 29.0. The decline in the number of red blood cells is paralleledby a reduction in the haemoglobin concentration from 13.0 to 8.8 g %. These declines are almost certainly at least partly related to the concomitant breakdown that takes place in the haemopoietic tissue of the fat column (Percy and Potter, 1976).

1.3. AIMS OF THIS INVESTIGATION

This investigation was initiated to utilise for a variety of different but interrelated studies the animals that were known to collect on the intake screens of the Oldbury-on-Severn Power Station. The particular significance of the sampling locality is that since it resides in the estuary it provides a source of upstream migrating lampreys prior to their entry into fresh water. This contrasts with the situation pertaining in most previous studies on the spawning run of River lampreys where the animals were caught after they had been in rivers for a variable length of time (e.g. Morris, 1958; Lanzing, 1959; Hardisty and Huggins, 1973; Larsen, 1973).

Regular comparable quantitative sampling was aimed at providing data on fluctuations in numbers which it was hoped could then be related to changes in environmental conditions. Such factors as discharge, lunar periodicity or temperature might then be demonstrated of importance in the initiation of the migration. Measurements of weights and lengths of animals in the estuary were made to ascertain the degree of heterogeneity of the population and

to determine whether there was a clearly defined group of praecox forms in addition to the typical larger individuals known to be present in relatively large numbers in the Severn river system (Hardisty and Huggins, 1973). The weights and lengths could also be compared with spawning populations to ascertain the degree of change during the migration while the measurement of body proportions would quantify where such changes were taking place. Likewise, gut, liver and gonad weights could be used to see how these organs undergo relative changes in size during the migration.

Another significant factor about an estuarine sampling site is that in view of the paucity of data on the ionic and osmotic concentration of the blood of lampreys in a marine environment, it can provide for analysis a reservoir of animals which have almost certainly only recently left full strength salt water. These data then provide the basis for comparisons with the ionic composition of tissues about which relatively little is known at any stage in the life cycle of lampreys.

2. MATERIALS AND METHODS

2.1. SAMPLING

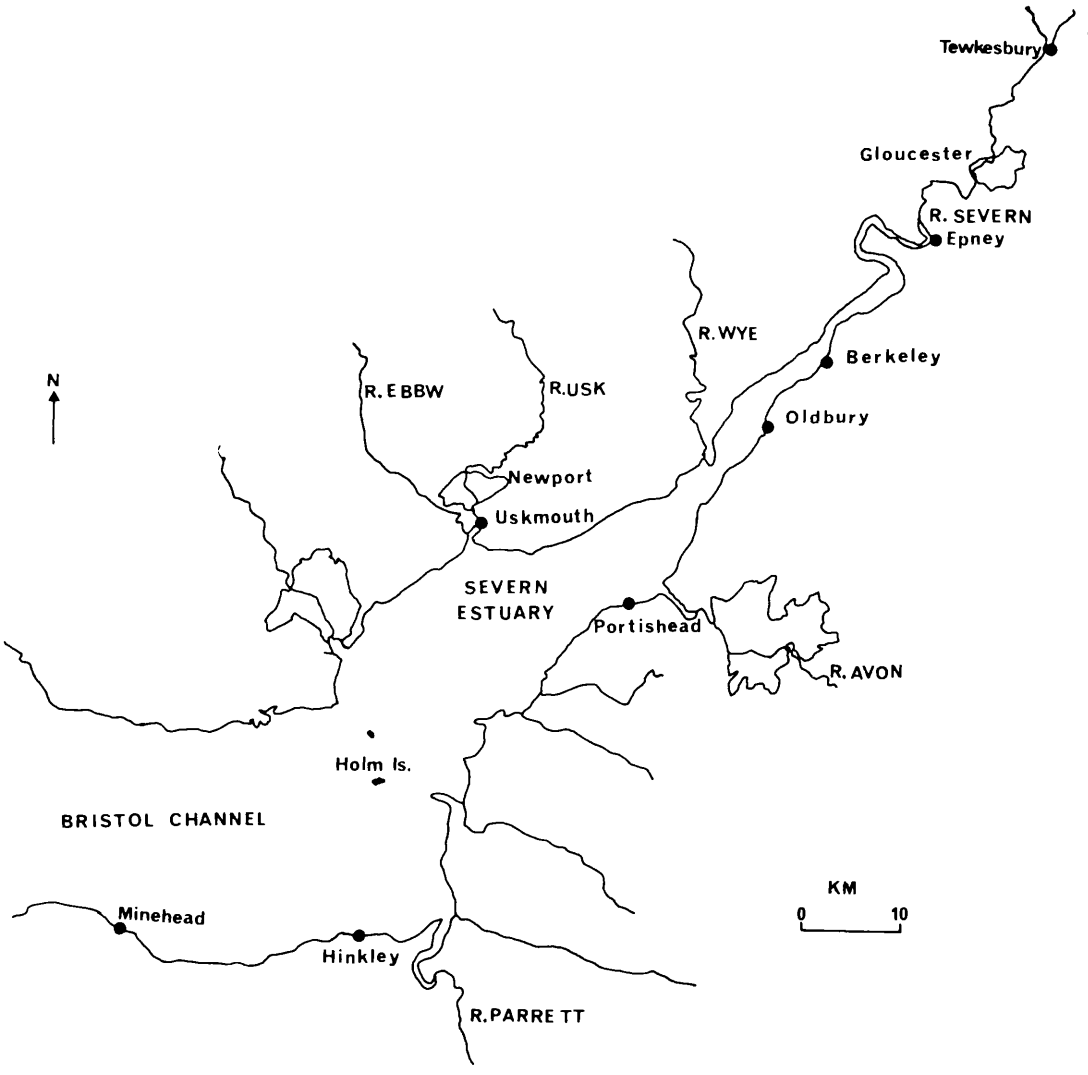
Upstream migrating River lampreys were collected at weekly intervals from the intake screens of the Nuclear Power Station at Oldbury-on-Severn (Figure 1). At this locality the river is approximately 1320 m wide at high tide but very much less at low tide. To retain water in the vicinity of the intakes at low tide, the region in front of the station has been deepened and surrounded by a wall. It is from the large reservoir thus formed that the cooling water for the reactors is drawn. Although the reservoir is isolated from the estuary at low tide, an exchange of water between the reservoir does occur during a period of 5-6 hours in each tidal cycle. At the time when water is passing over the wall animals can enter or leave the reservoir.

Prior to passing to the reactors, the cooling water flows first through a coarse screen consisting of vertical bars 25 cm apart before entering the centre of a 12.2 m diameter revolving drum containing a wire mesh of 1 cm (Plate 1 & Plate 2). Small trays (Plate 1 & Plate 2) on the inside surface of the drum collect the plant and animal material which is then flushed by a jet of water into a drain. The water is finally deposited in a skip prior to removal.

There are four drum screens, each of which is served by a separate pump. As all the pumps are not operational throughout the year, there are differences between the cumulative intake rates at different times. Thus, in the summer, the total amount of

FIGURE 1

A map of the River Severn, the Estuary and the Bristol Channel showing the location of the Oldbury and Uskmouth Power Stations and the position of Gloucester and Tewkesbury.


Tewkesbury
Gloucester
R. SEVERN
Epney
R. WYE
Berkeley
N
R. EBBW
R. USK
Oldbury
Newport
Uskmouth
SEVERN
ESTUARY
Portishead
R. AVON
Holm Is.
BRISTOL CHANNEL
KM
0
10
Minehead
Hinkley
R. PARRETT

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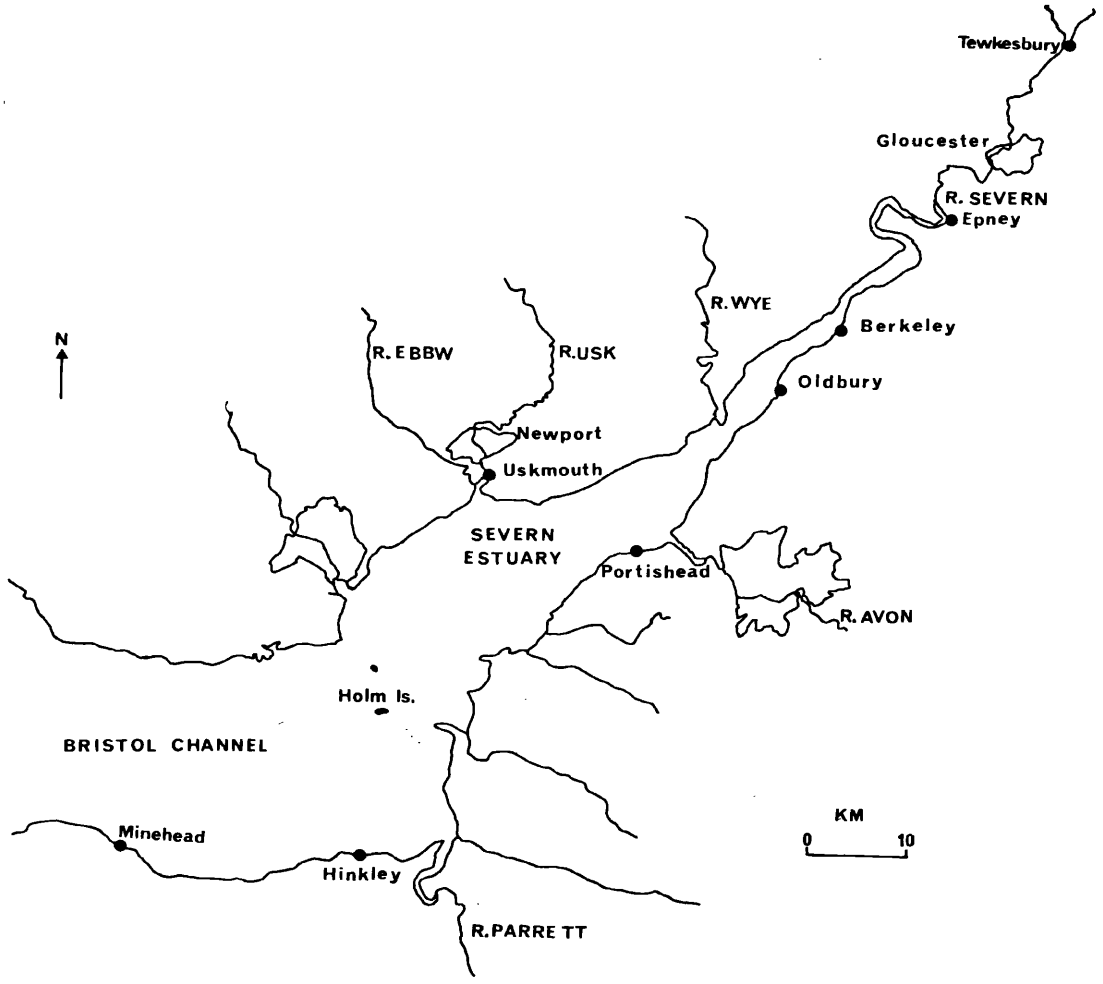

Tewkesbury
Gloucester
R. SEVERN
Epney
R. WYE
Berkeley
N
R. EBBW
R. USK
Oldbury
Newport
Uskmouth
SEVERN
ESTUARY
Portishead
R. AVON
Holm Is.
BRISTOL CHANNEL
KM
0
10
Minehead
Hinkley
R. PARRETT

PLATES 1 & 2

Screening mechanism of Oldbury Nuclear Power Station situated in the middle reaches of the Severn Estuary.

1

2

water pumped each 24 hours is 1310 million litres, whereas in the winter it is raised to 2074 million litres. Since the actual rate is always known and all the material from each of the screens over each 24 hour period is deposited in the same skip, the number of lampreys obtained can be expressed in terms of the same volume of pumped water. For convenience, the numbers have thus been expressed as the number of lampreys taken in the equivalent of 2074 million litres of water pumped over a 24 hour period. Since samples were always collected each Tuesday, and occasionally also on other days of the week, an indication of the relative size of monthly abundance could also be determined. Sampling commenced in September 1972 and was concluded in November 1976.

Collections of migrating River lampreys were also made at the Power Station located on the river Usk at a site only 0.5 km from the point at which it enters the Severn Estuary (Figure 1). This locality, which is situated 31.5 km further down the estuary than Oldbury, was sampled randomly and only during 1975 and 1976.

After their entry into fresh water in 1973-76, lampreys were collected from in front of the weir at Tewkesbury on the River Severn. These were held in the laboratory for a short period and then sexed, weighed, and measured in order to provide the data that could be compared with animals taken at Oldbury.

Sexually mature and spent River lampreys *(L. fluvatilis)* were also collected by hand in late March and early April from the River Teme at Tenbury Wells and from Chulmleigh on the River Taw in Devon.

The mean daily fresh water flow were taken from the Severn-Trent Water Authority readings taken at Gloucester, while the

temperatures came from the records of the Oldbury Power Station. The salinity data were kindly provided by Dr. P.N. Claridge who has recorded the values at Oldbury on each Tuesday since September 1974.

2.2. BODY MEASUREMENTS

The measurements of the lengths and body intervals of the animals were made on the left side of the body and recorded to the nearest mm. Body proportions (Figure 2) were then expressed as a percentage of the total body length.

Total length (TL): The distance from the anterior edge of the disc to the termination of the caudal fin.

Disc length (d): The length of the disc in its relaxed position.

Length of the preorbital region ($d-B_1$): The distance from the anterior edge of the disc to the anterior edge of the eye.

Diameter of the eye (O): Horizontal diameter of the eye.

Length of the prebranchial region ($d-B_1$): The distance from the anterior edge of the disc to the anterior edge of the first gill opening.

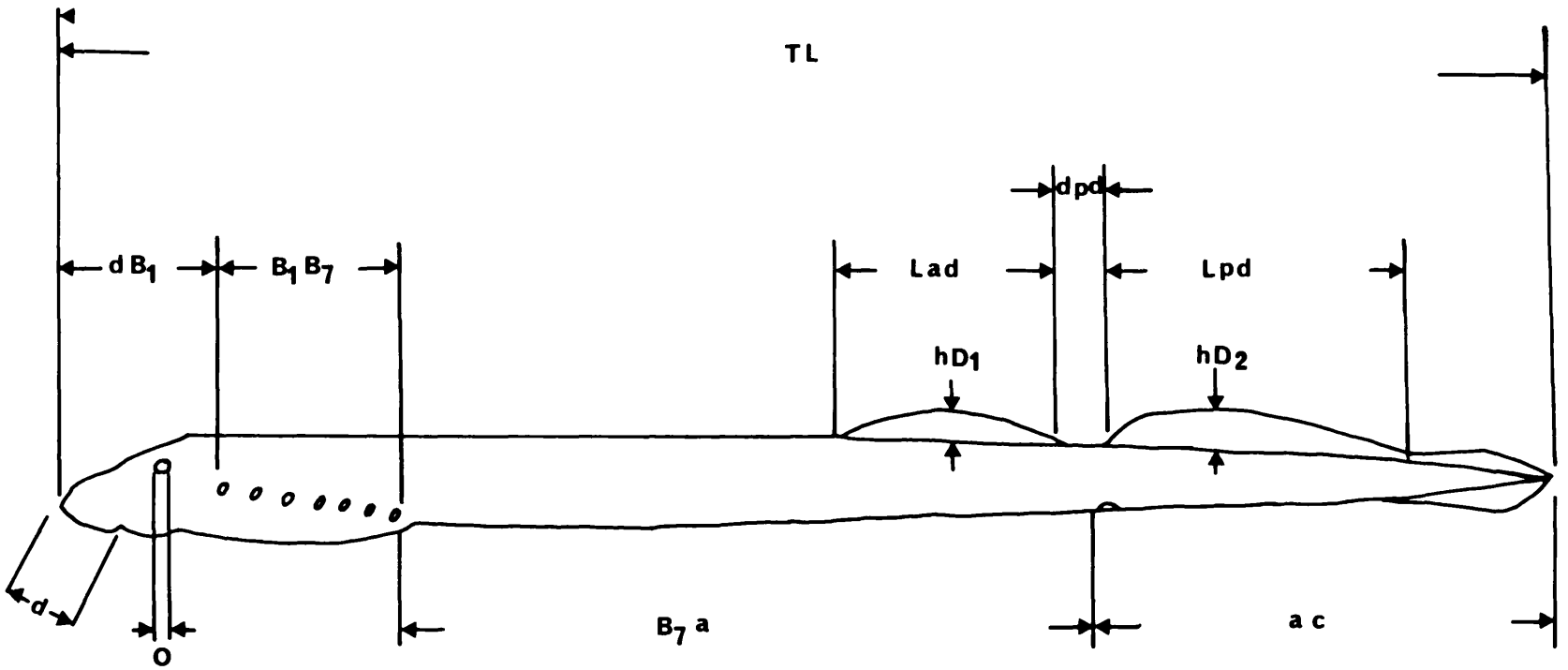
Branchial region ($B_1 - B_7$): The distance from the anterior edge of the first gill opening to the posterior edge of the seventh gill opening.

Trunk region ($B_7 - a$): The distance from the posterior edge of the seventh gill opening to the anterior edge of the cloaca.

Tail length (a-c): The distance from the anteror edge of the cloaca to the termination of the caudal fin.

FIGURE 2

A diagram of the body of an adult River lamprey, *Lampetra fluviatilis*, showing the various body proportions that were measured.


TL
dpd
dB1
B1B7
Lad
Lpd
hD1
hD2
d
O
B7a
ac

Length of anterior dorsal fin (lad): Horizontal length of the first dorsal fin along the body.

Length of posterior dorsal fin (Lpd): Horizontal length of the second dorsal fin along the body.

Height of first dorsal fin (hD_1): The maximum vertical height of the fin.

Height of second dorsal fin (hD_2): The maximum vertical height of the fin.

Length between the two dorsal fins (dpd): The horizontal length from the posterior end of the first dorsal fin to the anterior end of the second dorsal fin.

Position of the anterior dorsal fin (ppd): Anterior edge of the second dorsal fin.

Position of the posterior dorsal fin (ppd): Anterior edge of the second dorsal fin.

The body wet weight of the animal was recorded to the nearest 0.1 g. Condition factors (CF) were later calculated from the equation $\frac{W}{L^3} \times 10^6$ where W is the body weight in g and L the length in mm.

Each animal was cut open and its liver, gut, and gonad removed and then weighed to the nearest 0.1 g after first quickly and carefully removing the surface water with tissue paper. In the case of those early migrants, where there was still food in the gut, the gut contents were carefully squeezed out prior to weighing. The weights were expressed as a percentage of the total body wet weight.

2.3. HISTOLOGY AND MEASUREMENTS ON THE OVARY AND GUT

Portions of the mid-region of the ovaries and the gut were fixed in Bouin's. After embedding in paraffin, sections were cut at 7 μm and stained with Ehrlich's haematoxylin and eosin. The diameter of the oocytes given in the results is the mean of the maximum and minimum diameters measured in the above sections with a micrometer eye piece.

In order to ascertain when sexual maturity was reached in the praecox forms, and to determine the size and number of the eggs at this time, a number of the small animals caught in February and March were kept in laboratory aquaria whose temperature was maintained at $10 \pm 1^{\circ}C$. When the secondary sexual characters were fully developed, all the eggs were removed, weighed and placed in Gilson's fluid (Simpson, 1951). After 24 h the eggs were reweighed and a count made of the number in a subsample of approximately 1 g, thereby permitting an estimate of the total fecundity to be made. The mean diameter of at least 20 eggs from seven different praecox forms was determined with a dissecting microscope using a micrometer eye piece.

2.4. PHYSIOLOGICAL EXPERIMENTS

2.4.1 Animals

The live and healthy representatives of the samples brought back from Oldbury, Tewkesbury and Tenbury, together with animals held in the laboratory for several weeks, were used for the studies

on ionic regulation. In all cases the animals were acclimated to a temperature of 10 $\pm$ 1°C before experimentation. The water in which they were maintained was always well aerated and consisted either of brackish water taken from Oldbury at the time of capture, or of aged tap water in the case of animals caught in the river.

2.4.2. Preparation of Plasma and Measurement of Haematocrit and Concentration

After the animal had been anaesthetised in an 0.1 g l^{-1} solution of MS222 (Sandoz), blood was obtained by cardiac puncture and centrifuged for 5 mins at 3,000 rpm under liquid paraffin. The plasma was pipetted off and stored in sealed vials at -10°C until required.

Some of the blood collected by cardiac puncture was also used for measurements of haematocrit and haemoglobin concentration. For the former, blood was allowed to flow from the syringe into a heparinised capillary tube which was then centrifuged for 6 mins at 12,000 g in a microhaematocrit centrifuge. The packed cell volume was measured with a microhaematocrit reader, enabling its relative volume in the blood (i.e. haematocrit) to be estimated.

The haemoglobin concentration was determined by the method described by Drabkin (1935). 0.02 ml of blood were added to 4ml of Drabkin's reagent (0.0125 g KCN in 250 ml distilled water) and after thorough mixing allowed to stand for at least 5 mins. Its optical density, together with that of a cyanmethaemoglobin standard whose concentration was 57.2 mg 100 ml^{-1}, were then measured at 540 nm. The haemoglobin concentration could then be calculated from the equation

$$\text{Haemoglobin Conc.(g \%)} = \frac{\text{OD of test sample}}{\text{OD of standard}} \times \frac{\text{conc. of st. mg\% x 200}}{1000}$$

2.4.3. Preparation of Material for Measurement of Tissue Ions

After removing the skin, a narrow stip of parietal muscle weighing about 0.4 g was taken from a region below the first dorsal fin and placed in a stoppered vial. The tissue was freeze-dried overnight and re-weighed, after which 2 ml of 50% Analar HNO_3 was added. The vial containing the muscle was sealed and stored at room temperature for one week to assure complete digestion. Portions of the liver and gonads were also treated in the same way, as was a segment of the gut after it had been cut open and its internal surface cleaned by wiping and blotting with damp filter paper.

For the analysis of the electrolytes in the whole animal, the lamprey was wiped with tissue paper, weighed and then freeze dried. After re-weighing, 100 ml of 50% Analar HNO_3 were added to the material which had been placed in a beaker. It was then sealed with 'Parafilm' and left for at least one week.

2.4.4. Measurement of Osmotic Pressure and Ions

All glassware were washed in 'Decon', immersed overnight in an acid bath (25% conc. HNO_3) rinsed and left for 2-3 weeks in frequent changes of deionised water. The clean 'ion leached' glassware was then dried before use.

Osmotic Pressure

The method used for measurement of osmotic pressure is essentially the same as that described by Ramsay and Brown (1955). The sample is enclosed in a capillary tube and frozen before being placed in a bath of 30% alcohol at a lower temperature than the

expected thawing point. The bath is then warmed slowly until the disappearance of the last crystal of ice. A curve for a series of NaCl standards (0 - 600 mM/l) against their freezing point enabled the measurement of the osmotic pressure.

Sodium and Potassium

The sodium and potassium in plasma and tissue extracts, diluted in a known volume of deionised water, were measured using an Eel Flame Photometer. Since spectral interference has been reported between these two ions when one is at a very different concentration than the other (Cooke and Price, 1966), standards were prepared to take this effect into account. For sodium the maximum working standards were 0.36 mM l^{-1} while for potassium it was 0.096 mM l^{-1}. Plotting the reading for emission against the various sodium and potassium standards produced a linear relationship passing through zero in the first case but not in the second.

Calcium and Magnesium

Atomic absorption spectrophotometry using a Unicam SP90 gave highly reproducible results both for tissue and for plasma. This technique is particularly useful for magnesium as this element exhibits the greatest sensitivity of all in atomic absorption studies. As protein has been reputed to interfere with magnesium determinations (Alcock and McIntyre, 1966), 0.75% EDTA was added instead of deionised water in order to dilute the plasma and tissue extracts (Cook *et al*, 1966). This has the effect of releasing the magnesium by breaking the protein magnesium bond. As high phosphate levels have been widely reported to have a depressant effect on calcium estimation (Bianchi, 1968), 0.75% EDTA was added to the plasma and tissue extracts used for

measuring this ion. The standards were also made up in 0.75% EDTA. The maximum working solutions for Ca^{++} were 0.15 mM l^{-1} and for Mg^{++} 0.07 mM l^{-1}. With magnesium, a straight line was obtained with the different saline standard solutions, whereas a slight curve towards the concentration axis was produced with calcium.

Chloride

Chloride in the plasma and tissue extracts was determined electrometrically (Cotlove, 1964) using a CMT10 chloride titrator (Radiometer Copenhagen). This technique gave highly reproducible results ($\pm$ 2%) and proved very sensitive even with samples having a total chloride content as small as 1.0 µm chloride. All samples were titrated in a media of saturated potassium sulphate containing 0.4 M sulphuric acid and a few drops of 0.1 g gelatin dissolved in 100 ml of 0.4 M sulphuric acid.

Inorganic Phosphorus

Inorganic phosphorus was estimated using Phosphorus Auto/stat TM Kit (Pierce). With this technique the colour development and the determination of absorbance takes place entirely in the acidic solution which is added (Raabe, 1955; Henry, 1964). An advantage is that the protein is neither precipitated nor removed at any step, but is held in solution by the use of a surfactant. Further advantages of the technique are that it uses only a single working reagent and conforms with Beer's Law over the range 0-15 mg inorganic phosphorus l^{-1}.

0.1 ml of sample was added to 3.0 ml of the working reagent the latter being prepared by mixing one volume of molybdate reagent containing a catalyst (this consisting of 1.0% ammonium paramolybdate,

heptahydrate and 2.12% sulphuric acid) together with 10 volumes of the reducing reagent (this consisting of 1.0% sodium acetate trihydrate, 2.6% acetic acid, 1.0% sodium metabisulphite, 0.5% p. methylammoniumphenol sulphate, together with a surfactant). The test mixture was allowed to stand at room temperature for a period of 30 mins, during which time the molybdenum colour develops, and is read at 690 nm using an SP 90 Unicam spectrophotometer with a standard of 5.0 mg 100 ml^{-1} (0.22% potassium dihydrogen phosphate in dilute HCl).

2.4.5. Measurement of Inulin Space

^{14}C labelled inulin (Radiochemical Centre) was used for measurements of the extracellular space. The animal was anaesthetised and then injected intraperitoneally with 0.1 ml (0.5 μ Ci ml^{-1}) of ^{14}C inulin in an isotonic saline solution (consisting of 5.5 gm l^{-1} NaCl, 0.12 g l^{-1} $CaCl_2$, 0.14 g l^{-1} KCl, 0.2 Na_2PO_4.
The animal was transferred to aquaria containing one litre of well aerated water and kept for a known time at a temperature of 10°C. For estimation of the amount of ^{14}C inulin in the plasma, 0.1 ml of the plasma was added to 10 ml of Unisolve 1 emulsifier (Koch-Light) and assayed in a liquid scintillation counter. 1 ml of bathing media was then counted in the same manner, and in both cases the quench correction was made using the internal standards method (Rogers and Moran, 1966). The extracellular space (V) was calculated from the equation

$$V = \frac{In_{ti} - In_{te}}{In_p}$$

In_{ti} = Total amount of inulin injected into the animal (count min^{-1} ml^{-1})

In_{te} = Total amount of inulin in bathing fluid after known time (count min^{-1} ml^{-1})

In_{p} = Total amount of inulin in the plasma (count min^{-1} ml^{-1}).

2.4.6. Measurement of Testosterone and Progesterone

Radioimmunoassay using commercial kits (RIA TEK ICN, Portland, Oregon) was employed to estimate testosterone and progesterone in plasma pooled each month from 8-15 typical forms of *L. fluviatilis*. RIA was also performed on the sera of spawning Sea lampreys which had been collected from the River Tawe.

3. RESULTS

3.1. TIMING OF THE UPSTREAM MIGRATION

Although upstream migrants were caught at Oldbury in some years as early as August and one animal was observed at the Power Station in July, the spawning-run animals did not start appearing in any numbers in the Severn Estuary until rather later (Figures 3 & 4). Thus, the first real influx occurred in October in the seasons of 1973/74, 1974/75 and 1975/76, and in November and September of 1972/73 and 1976/77 respectively. It should be noted that graphs for this last season are not presented as this spawning run is still in progress. Despite the fact that there was considerable variability in numbers between successive weekly samples, the monthly catches showed a distinctly seasonal pattern in most years. Thus, although the maximum numbers in a 24 h sample occurred in January of 1972/73, peak values in 1973/74, 1974/75 and 1975/76 were found in November.

These differences in the timing of the spawning migration in different years can be considered in relation to the environmental conditions prevailing in the different seasons (Figures 3 & 4). In this context, it is therefore almost certainly significant that the relatively very late run of 1972/73 occurred after a long dry period in the autumn and winter during which discharge rates were low. Furthermore, the much earlier run of 1976/77 was initiated after the most exceptionally high and continuous freshwater discharge rates encountered in the autumn of any of the five years comprising this investigation. There would appear therefore to be little doubt that

FIGURE 3

The number of lampreys collected in 24 hour samples from the Oldbury Power Station during the spawning run seasons of 1972/73 and 1973/74. Data has been corrected to correspond to the minimum intake volume found during the year, namely 2.074×10^9 l. Also included are the mean daily water temperatures at Oldbury and the water discharge measured in the River Severn at Gloucester.

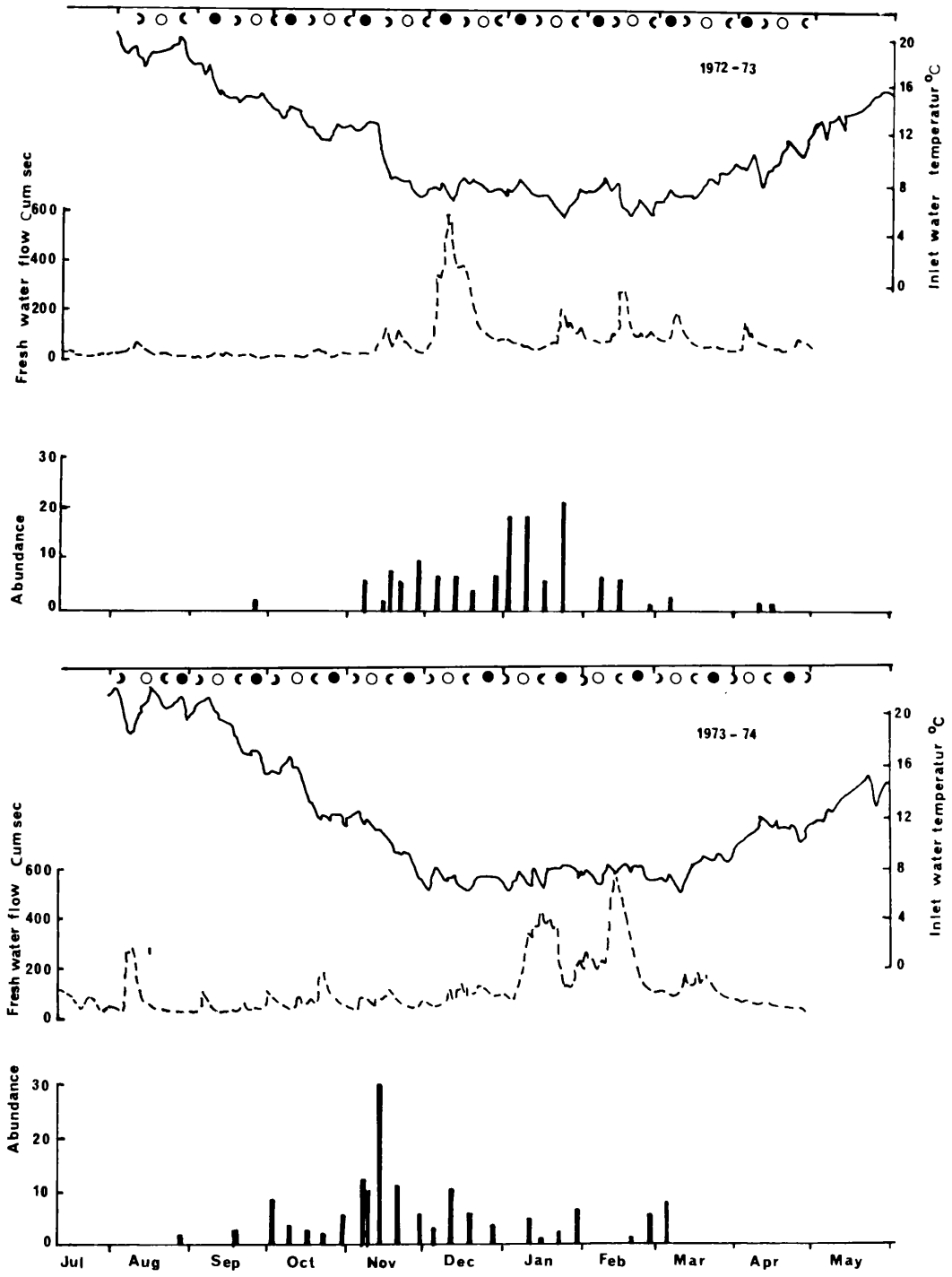
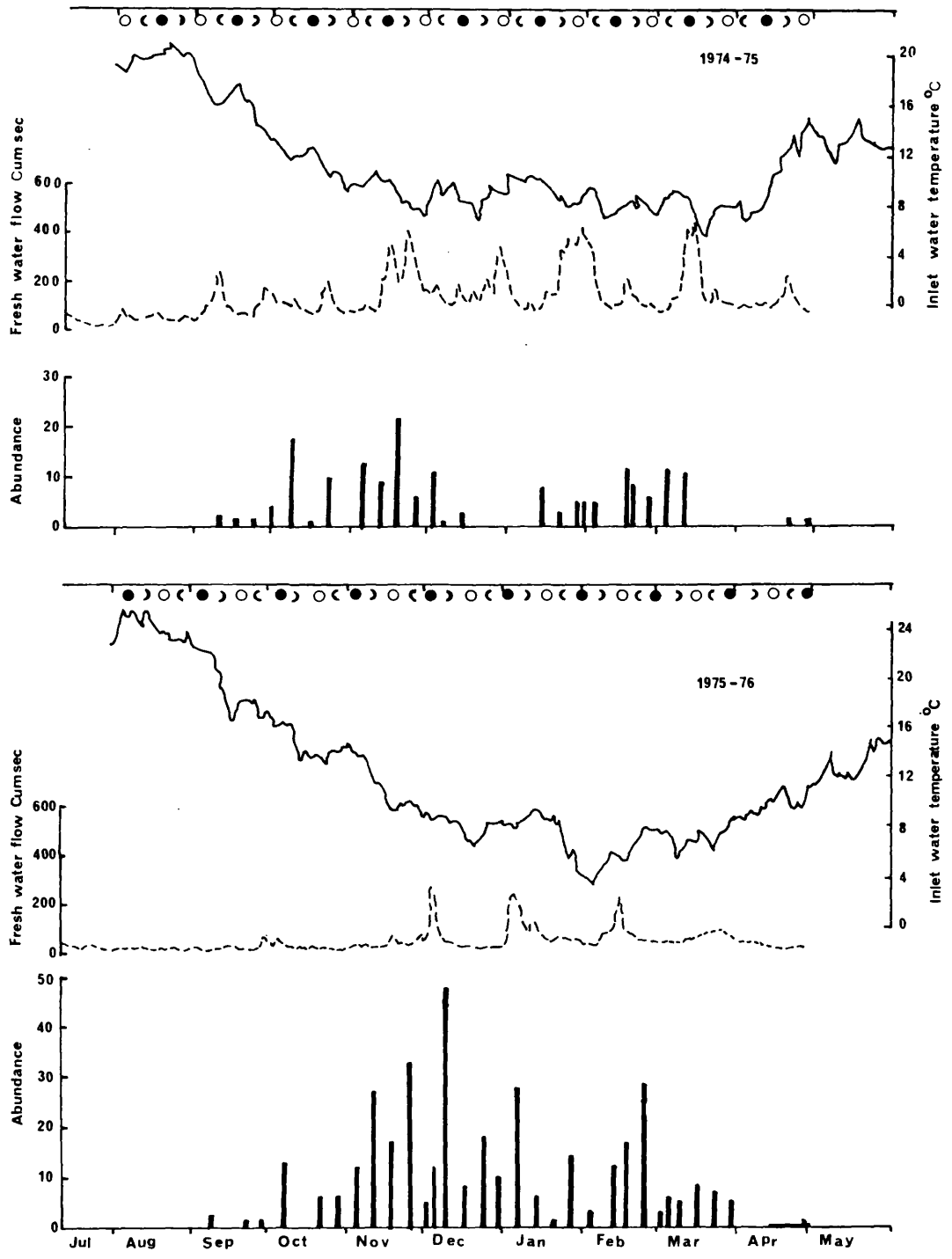

1972 - 73
Fresh water flow Cum sec
Inlet water temperatur °C
Abundance
1973 - 74
Fresh water flow Cum sec
Inlet water temperatur °C
Abundance
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May

FIGURE 4

The number of lampreys collected in 24 hour samples from the Oldbury Power Station during the spawning run seasons of 1974/75 and 1975/76. Data has been corrected to correspond to the minimum intake volume found during the year, namely 2.074×10^9 l. Also included are the mean daily water temperatures at Oldbury and the water discharge measured in the River Severn at Gloucester.


1974 - 75
Fresh water flow Cumsec
600
400
200
0
Inlet water temperature °C
20
16
12
8
4
0
Abundance
30
20
10
0
1975 - 76
Fresh water flow Cumsec
600
400
200
0
Inlet water temperature °C
24
20
16
12
8
4
0
Abundance
50
40
30
20
10
0
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May

increased discharge from the river into the estuary is a major factor in initiating the movement from the sea into the rivers. This view is also supported by considering intermittent increases in discharge in relation to the numbers caught at different times during 1975/76, the season which yielded far higher numbers than any other year (Figure 4). For example, increases in number in the individual samples from the estuary at Oldbury often followed soon after there had been an increased rate of discharge in the river at Gloucester. It should be noted that because of the fact that these discharge readings were made in the River Severn some 35 km from Oldbury, freshwater would reach the lower parts of the estuary and Bristol Channel a little later than they are shown on the Figures.

After allowing for a slight time lag in freshwater discharge values between Gloucester and Oldbury, it is clear from the figures that an increased discharge does not necessarily bring about a decline in temperature. Water discharge would thus appear to be of more importance in initiating the movement of River lampreys into the estuary than temperature changes, although it is worth noting that while the first real influx of animals into the estuary occurred at different times, it always took place at about the same temperature, namely 14^{o}C (Figures 3 and 4).

There were no clear indications that lunar periodicity exerted a secondary effect on migratory patterns although in 1975/76 there was some very limited evidence that the numbers tended to be lower at or near the time of a full moon (Figure 4).

3.2. LENGTH AND WEIGHT-FREQUENCY CURVES

The length-frequency curves for animals caught at Oldbury between October and December displayed a similar pattern in each of the four years investigated (Figures 5 & 6). Thus, the distributions showed a strong tendency to be unimodal with peaks generally between approximately 280 and 320 mm. While the shape of the distributions, particularly for November and December, indicates that the population is homogeneous at these times, the samples from January onwards clearly showed a second peak in the length-frequency distributions. This second peak occurring at a length of approximately 235 mm, corresponds to a group which becomes increasingly predominant through to March, and which is occasionally also represented by small numbers as late as April. These data thus clearly demonstrate that two different size groups enter the estuary and that their period of peak abundance differs.

In an attempt to differentiate between the groups, animals caught in each month were separated into those longer and shorter than the minimum length found in the length-frequency curves between the two separate peaks, a point which in most cases is relatively well defined (Figures 5 & 6). These data then permitted an estimate to be made of the numbers and the ratio of the larger and smaller individuals of *L. fluviatilis* in each monthly catch, the two size categories corresponding respectively to what Berg (1948) has described as the typical and praecox forms (Table 1-3). Furthermore, the actual numbers of animals caught confirm that the maximum abundance of the typical form is generally reached at Oldbury in November (Table 1) whereas in the praecox form it occurs in either February or March (Table 2). It is also clear that during each spawning run season the number of

FIGURE 5

Length-frequency curves for River lampreys caught each month at the Oldbury Power Station during the 1972/73 and 1973/74 seasons. Data have been smoothed by a moving average of 25 mm.

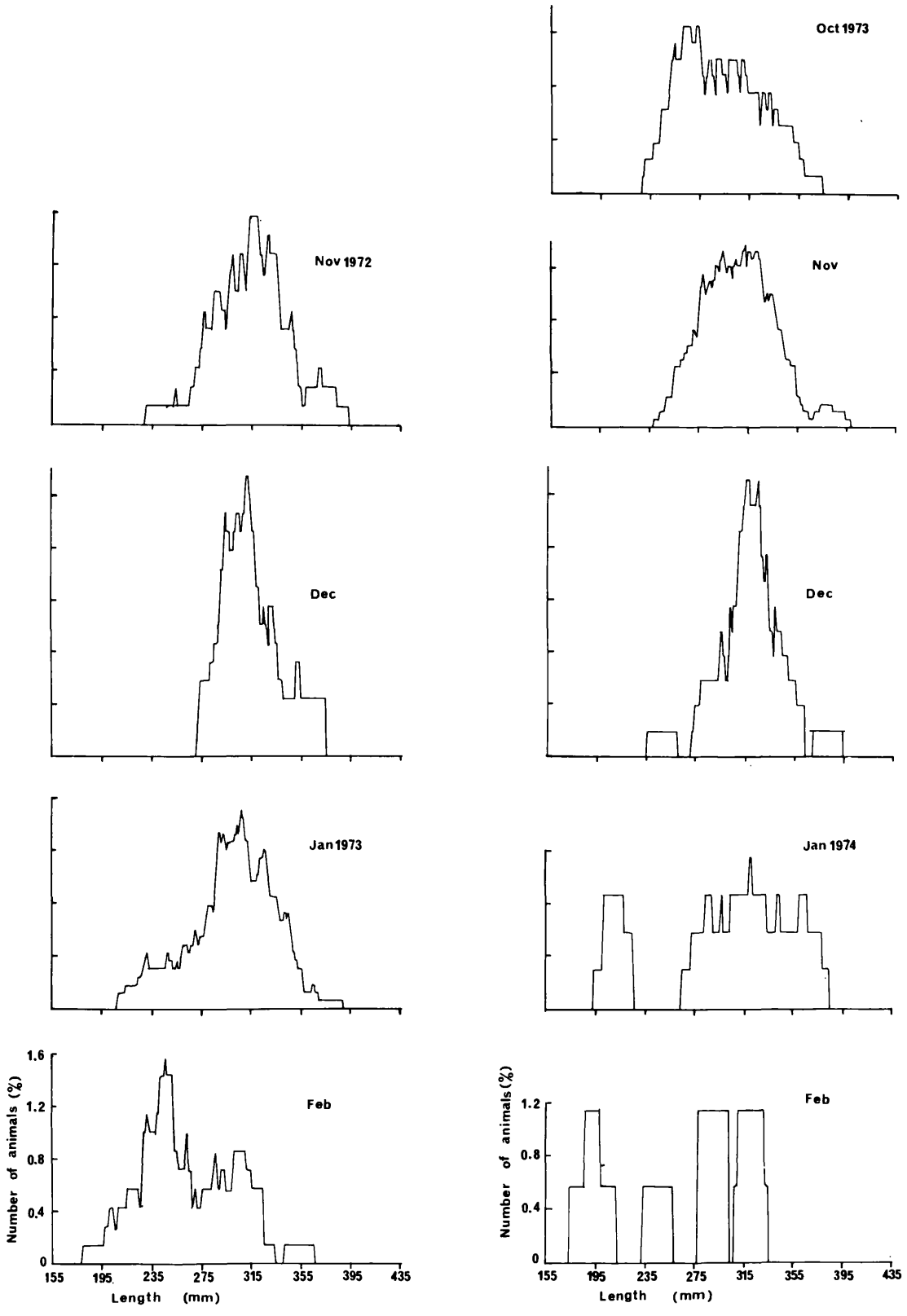

Oct 1973
Nov 1972
Nov
Dec
Dec
Jan 1973
Jan 1974
Feb
Feb
Number of animals (%)
1.6
1.2
0.8
0.4
0
155
195
235
275
315
355
395
435
Length (mm)

FIGURE 6

Length-frequency curves for River lampreys caught each month at the Oldbury Power Station during the 1974/75 and 1975/76 seasons. Data have been smoothed by a moving average of 25 mm.

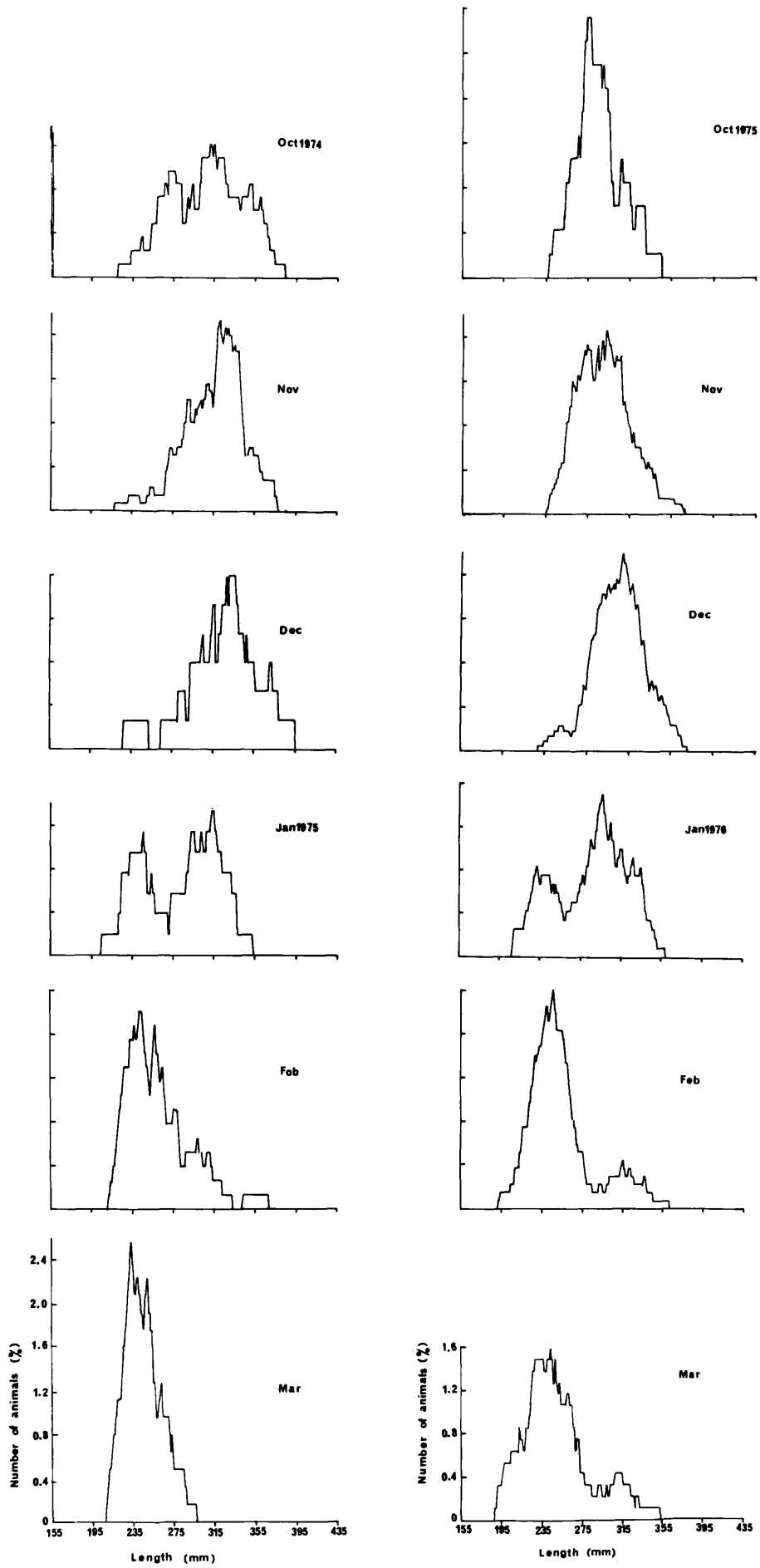

Oct1974
Oct1975
Nov
Nov
Dec
Dec
Jan1975
Jan1976
Fob
Feb
Mar
Mar
Number of animals (%)
2.4
2.0
1.6
1.2
0.8
0.4
0
155
195
235
275
315
355
395
435
Length (mm)

TABLE 1

The number of typical forms collected from the Oldbury Power Station between August 1972 and April 1976

MONTH	1972/73		1973/74		1974/75		1975/76		TOTAL NUMBERS		SEX RATIO FOR MONTHS	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
August	-	-	1	-	-	-	-	-	1	-	1 :	0
September	1	-	-	1	3	1	1	3	5	5	1 :	1.00
October	-	-	12	14	16	17	12	5	40	36	1 :	0.90
November	14	13	30	39	24	31	38	51	106	134	1 :	1.26
December	11	14	9	11	9	4	38	48	67	77	1 :	1.15
January	32	23	6	4	6	8	13	22	57	57	1 :	1.00
February	3	3	3	1	5	2	11	4	22	10	1 :	0.45
March	1	-	2	-	1	-	9	7	13	7	1 :	0.54
April	1	-	-	-	-	-	-	-	1	-	1 :	0.00
TOTAL	63	53	62	70	64	63	122	142	311	326	-	
Sex Ratio For Years	1 : 0.84		1 : 1.13		1 : 0.98		1 : 1.16		1 : 1.05		-	

TABLE 2

The number of praecox forms collected from the Oldbury Power Station between August 1972 and April 1976

MONTH	1972/73 ♀	1972/73 ♂	1973/74 ♀	1973/74 ♂	1974/75 ♀	1974/75 ♂	1975/76 ♀	1975/76 ♂	TOTAL NUMBERS ♀	TOTAL NUMBERS ♂	SEX RATIO FOR MONTHS ♀ : ♂
December	-	-	-	1	1	-	1	1	2	2	1 : 1.00
January	4	6	1	2	1	4	6	4	12	16	1 : 1.33
February	5	3	1	2	11	11	19	20	38	36	1 : 0.95
March	2	-	3	3	9	21	14	6	28	30	1 : 1.07
April	-	-	-	-	-	2	1	-	1	2	1 : 2.00
TOTAL	11	9	5	8	22	38	41	31	81	86	-
Sex Ratio For Years	1 : 0.82		1 : 1.60		1 : 1.72		1 : 0.76		1 : 1.06		-

TABLE 3

The percentage of typical forms in the samples taken from Oldbury

MONTH	1972/73		1973/74		1974/75		1975/76		TOTAL	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
August	-	-	100	-	-	-	-	-	100	-
September	100	-	-	100	100	100	100	100	100	100
October	-	-	100	100	100	100	100	100	100	100
November	100	100	100	100	100	100	100	100	100	100
December	100	100	100	91.7	90.0	100	97.4	98.0	97.1	97.5
January	88.9	79.3	85.7	66.7	85.7	66.7	68.4	84.6	82.6	78.1
February	37.5	50	75.0	33.3	31.3	13.4	36.7	16.7	36.7	21.7
March	33.3	0	40.0	0	10.0	0	39.1	53.8	31.7	18.9
April	-	100	-	-	0	-	-	0	50.0	0

typical forms predominate over the praecox forms. For example, in 1975/76 a total of 264 larger animals were caught compared with 72 of the smaller individuals, the overall percentages for the four years being 79.2 and 20.8% respectively.

3.3. CHARACTERISTICS OF TYPICAL FORMS DURING THEIR PEAK ABUNDANCE

Although, in every one of eleven collections made between October and December the females were larger than the males (Appendix 1-8) in none of the individual monthly samples were the means significantly different ($p < 0.05$). After pooling the data for the three years 1973/74, 1974/75 and 1975/76, when animals entered the estuary at a similar time, the lengths and the weight of the males and the females each showed the very clear distinction found between the typical and praecox forms in pooled data for both sexes in each of the different seasons (Figure 7). This distinction is also clearly seen in the weight-frequency curves for different years and the separate pooled data for males and females from all years (Figures, 8, 9 & 10).

One consistent pattern to emerge from the data for the October to December period in 1973/74, 1974/75 and 1975/76 is that without exception, a slight increase occurred in the modal and mean length of the typical males in each successive month. For example, in October, November and December of 1975, the mean lengths were 272.2, 287.3 and 301.5 mm (Appendix 8). Although a similar pattern was sometimes observed in the females, it was neither as marked nor as consistent (Appendix 1-8). When the data for the three years were pooled in fact the means for females caught in October, November and December and also January were similar (Figure 11). That there was a

FIGURE 7

Length-frequency curves for male and female River lampreys caught each month at the Oldbury Power Station between October and March of the 1973/74, 1974/75 and 1975/76 seasons. Data have been smoothed by a moving average of 25 mm.

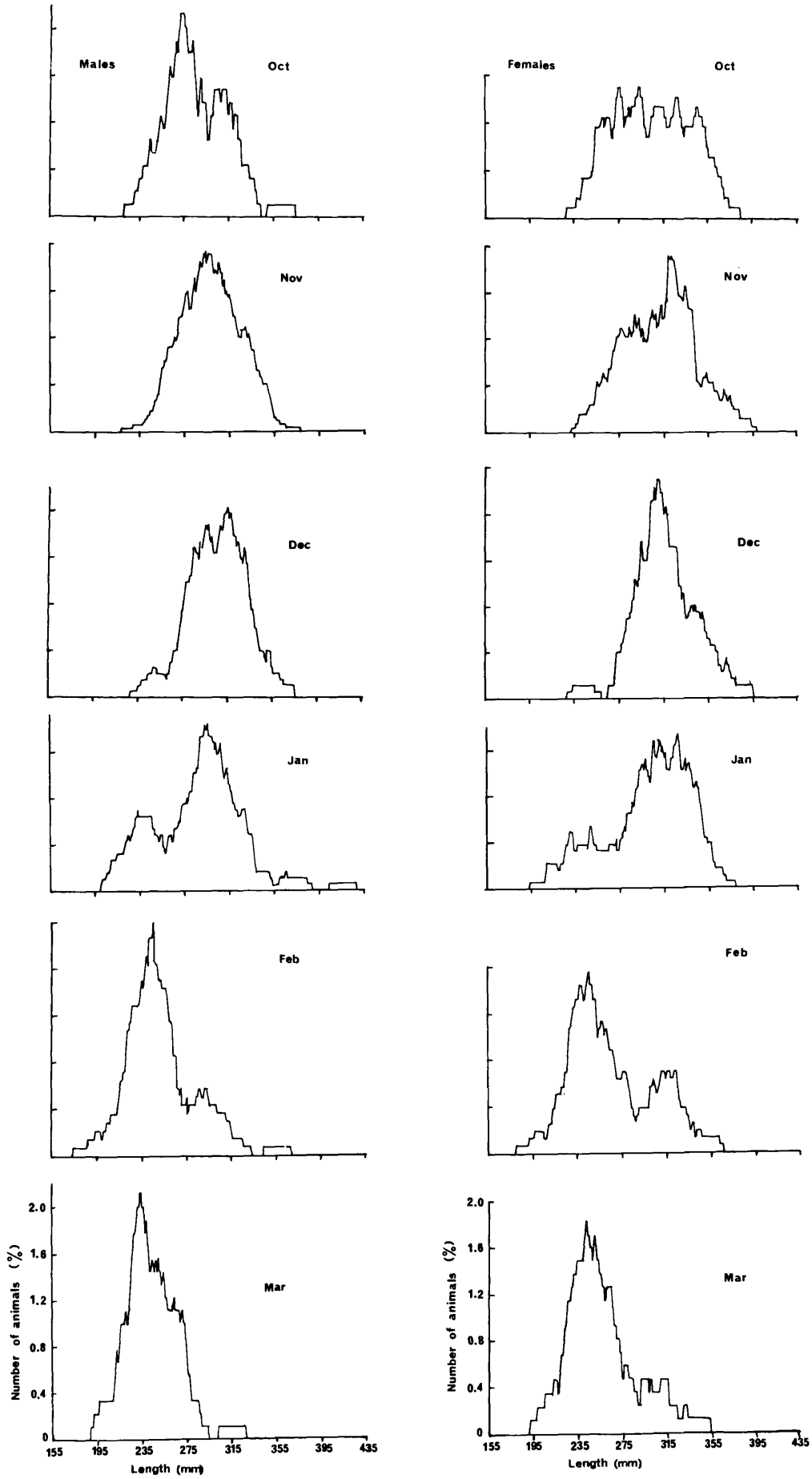

Males
Oct
Females
Oct
Nov
Nov
Dec
Dec
Jan
Jan
Feb
Feb
Mar
Mar
Number of animals (%)
2.0
1.6
1.2
0.8
0.4
0
155
195
235
275
315
355
395
435
Length (mm)

FIGURE 8

Weight-frequency curves for River lampreys caught each month at Oldbury Power Station during the 1972/73 and 1973/74 seasons. Data have been smoothed by a moving average of 7 g.

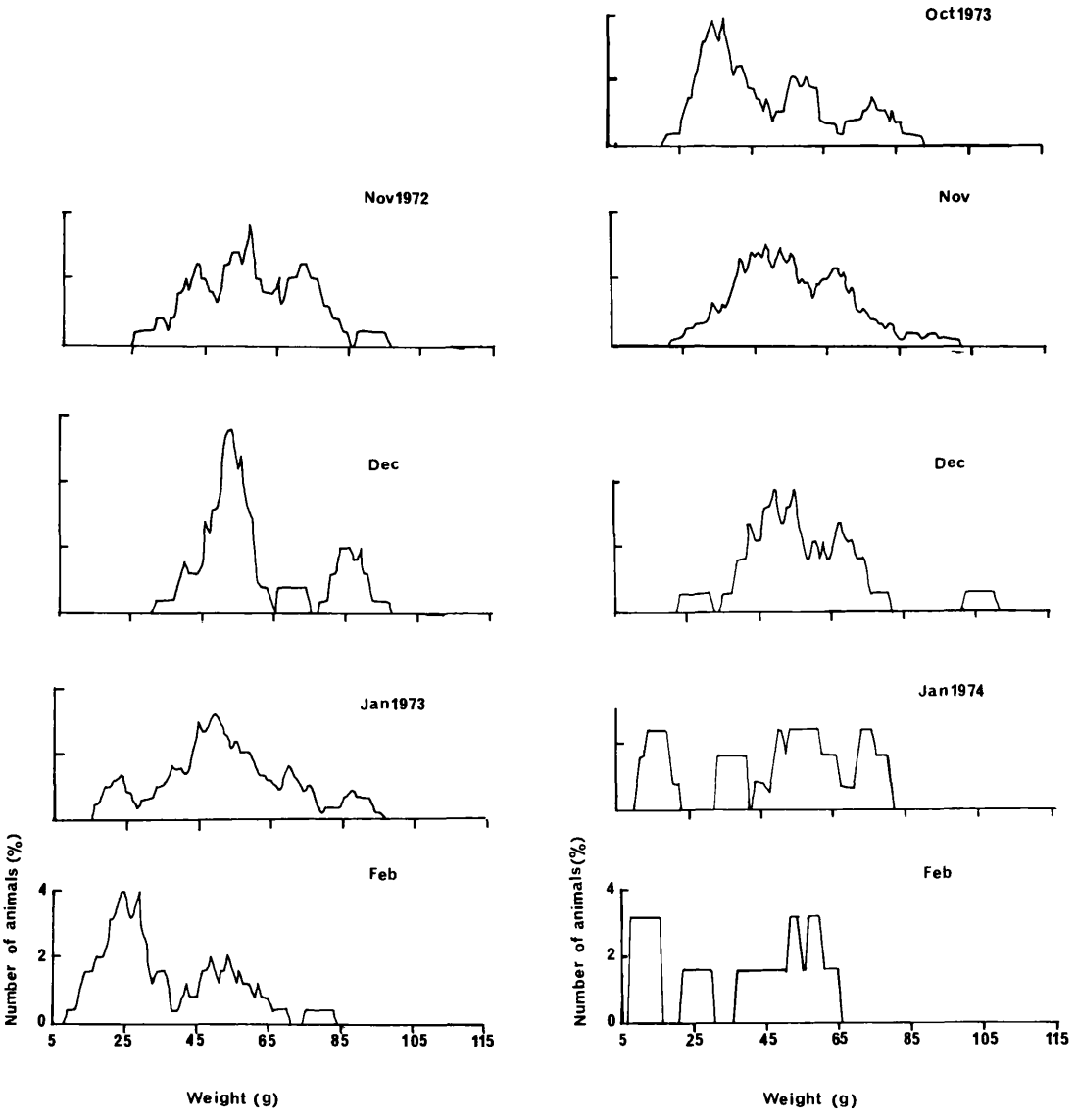

Oct1973
Nov1972
Nov
Dec
Dec
Jan1973
Jan1974
Feb
Feb
Number of animals (%)
Number of animals (%)
0
2
4
5
25
45
65
85
105
115
Weight (g)
Weight (g)

FIGURE 9

Weight-frequency curves for River lampreys caught each month at Oldbury Power Station during the 1974/75 and 1975/76 seasons. Data have been smoothed by a moving average of 7 g.

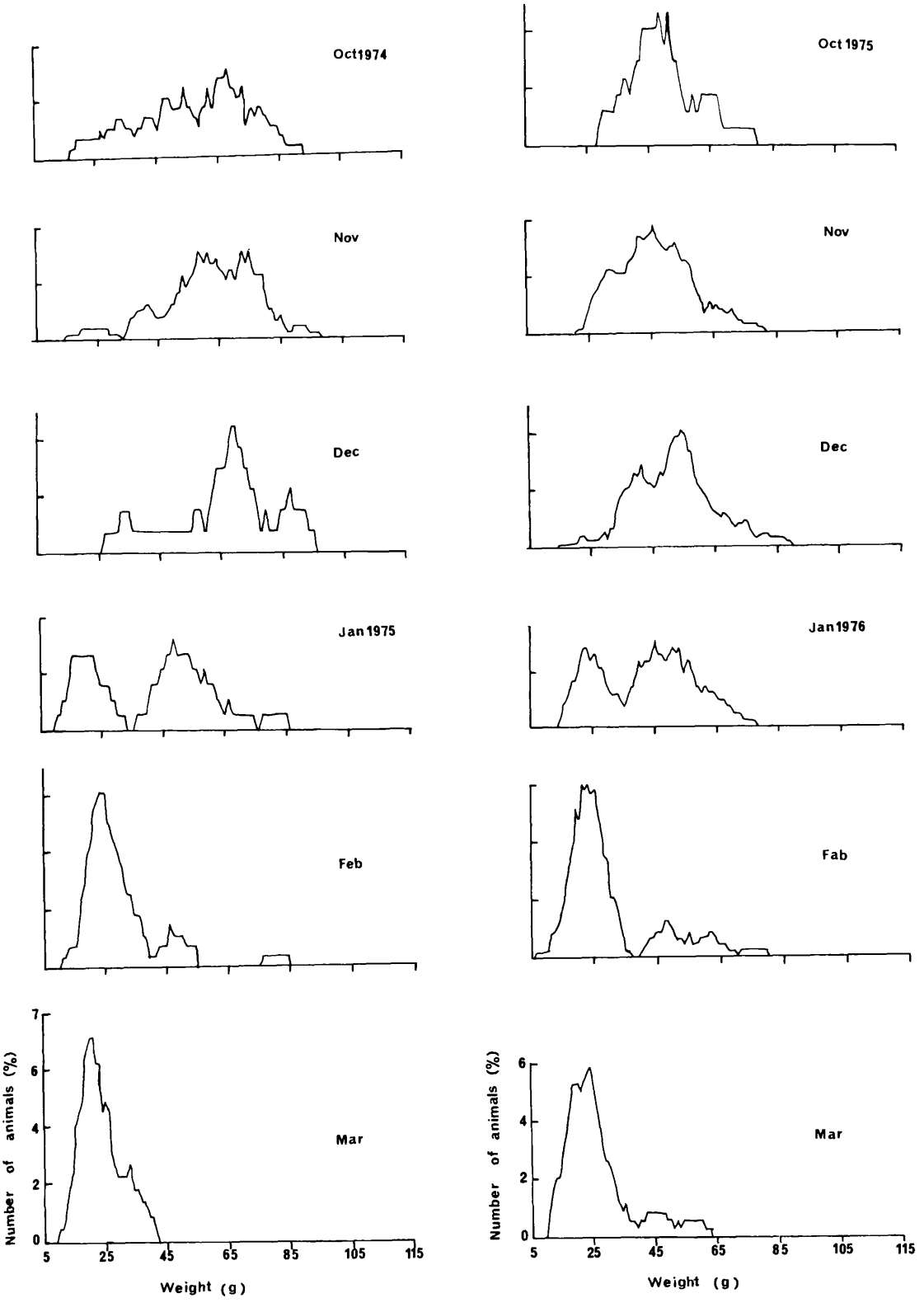
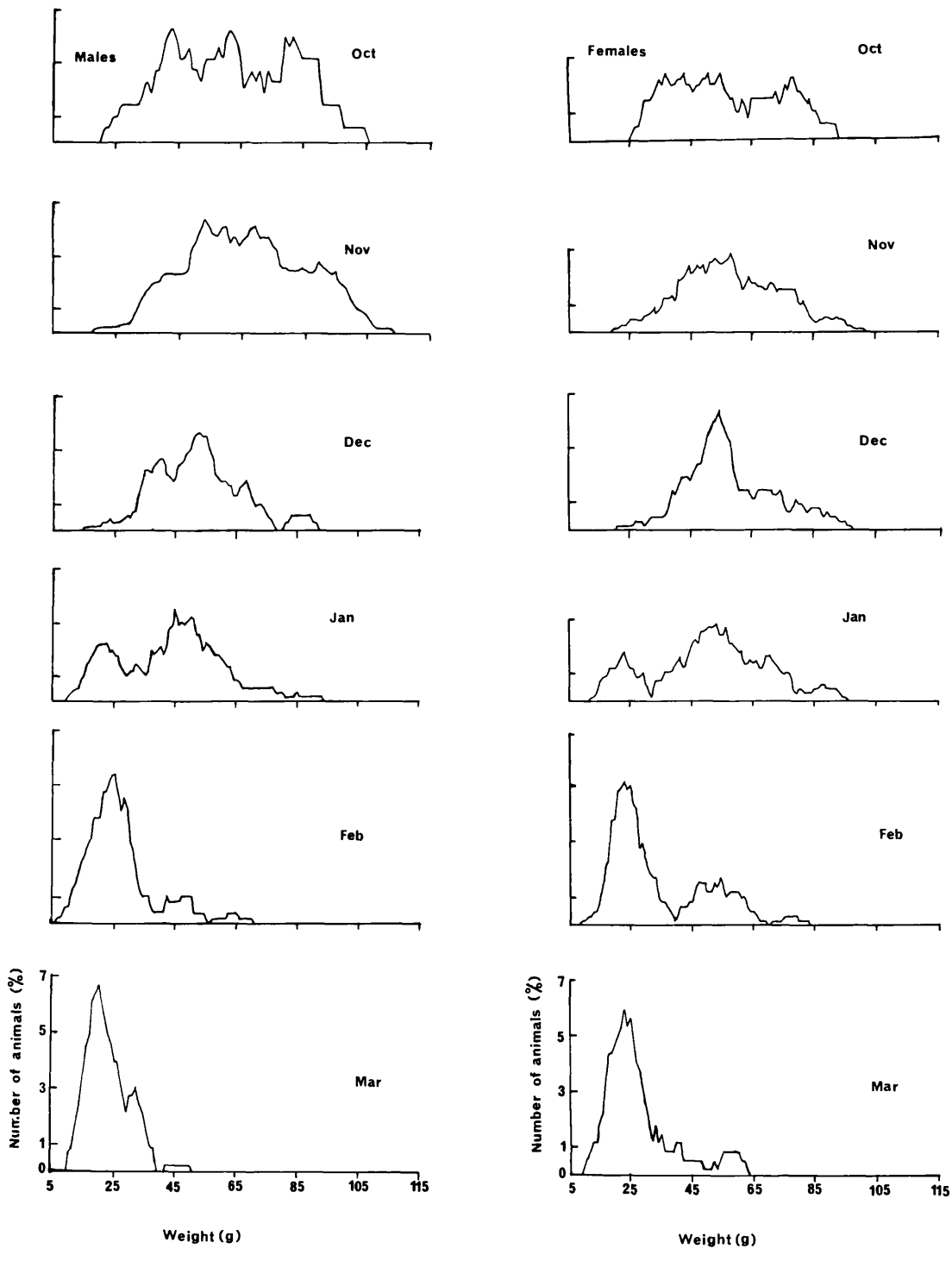

Oct1974
Oct 1975
Nov
Nov
Dec
Dec
Jan1975
Jan1976
Feb
Fab
Mar
Mar
Number of animals (%)
Number of animals (%)
Weight (g)
Weight (g)
0
2
4
6
7
5
25
45
65
85
105
115

FIGURE 10

Weight-frequency curves for male and female River lampreys caught each month at Oldbury Power Station between October and March of the 1973/74, 1974/75 and 1975/76 seasons. Data have been smoothed by a moving average of 7 g.


Males
Oct
Nov
Dec
Jan
Feb
Mar
Females
Oct
Nov
Dec
Jan
Feb
Mar
Number of animals (%)
0
1
3
5
7
5
25
45
65
85
105
115
Weight (g)
Number of animals (%)
0
1
3
5
7
5
25
45
65
85
105
115
Weight (g)

very small decline in November and not a more pronounced rise in December can be attributed to the effects of small variations between years in the time of entry of the majority of the first migrants into the estuary and also to the relative numbers and the size of the individuals caught in different years. The situation in females can be compared, however, with pooled data for males for the three years in which the mean still clearly shows an increase in each successive month between October and January (Figure 11).

The pattern exhibited in the pooled data for the mean weights of typical males between October and January varied little and therefore, did not follow quite the same pattern as was exhibited by their lengths (Figure 11). Furthermore, in females the weight in pooled data actually declined between October and January whereas the mean length for the same animals remained similar (Figure 11).

The ratio of females to males ranged from 1:0.84 in 1972/73 to 1:1.16 in 1975/76, the overall for the four years being 1:1.05.

Turning to the condition factor, reflecting the relationship between weight and length, this can be seen to vary during peak abundance from values as high as 2.17 in males in October 1975 to as low as 1.71 in December 1975 (Appendix 1-8). An overall decline during the sampling period is illustrated by the pattern shown in the pooled data for different years (Figure 12).

In contrast to the pattern in condition factors, the gonadosomic ratio increased over the same sampling period (Appendix 1-8). Thus, in 1975, the ratios in October, November and December were 1.96, 4.09 and 4.83 in males and 6.61, 7.37 and 7.55% in females. In this case, and as has also been found by Larsen (1973) in Baltic populations of the River lamprey, the relative weight of the ovary was significantly

FIGURE 11

The mean length and weight of the typical forms collected from the Oldbury Power Station between October 1973 and March 1976. The vertical line represents 95% confidence limits on either side of the mean, these being included when the sampling size was greater than 11.

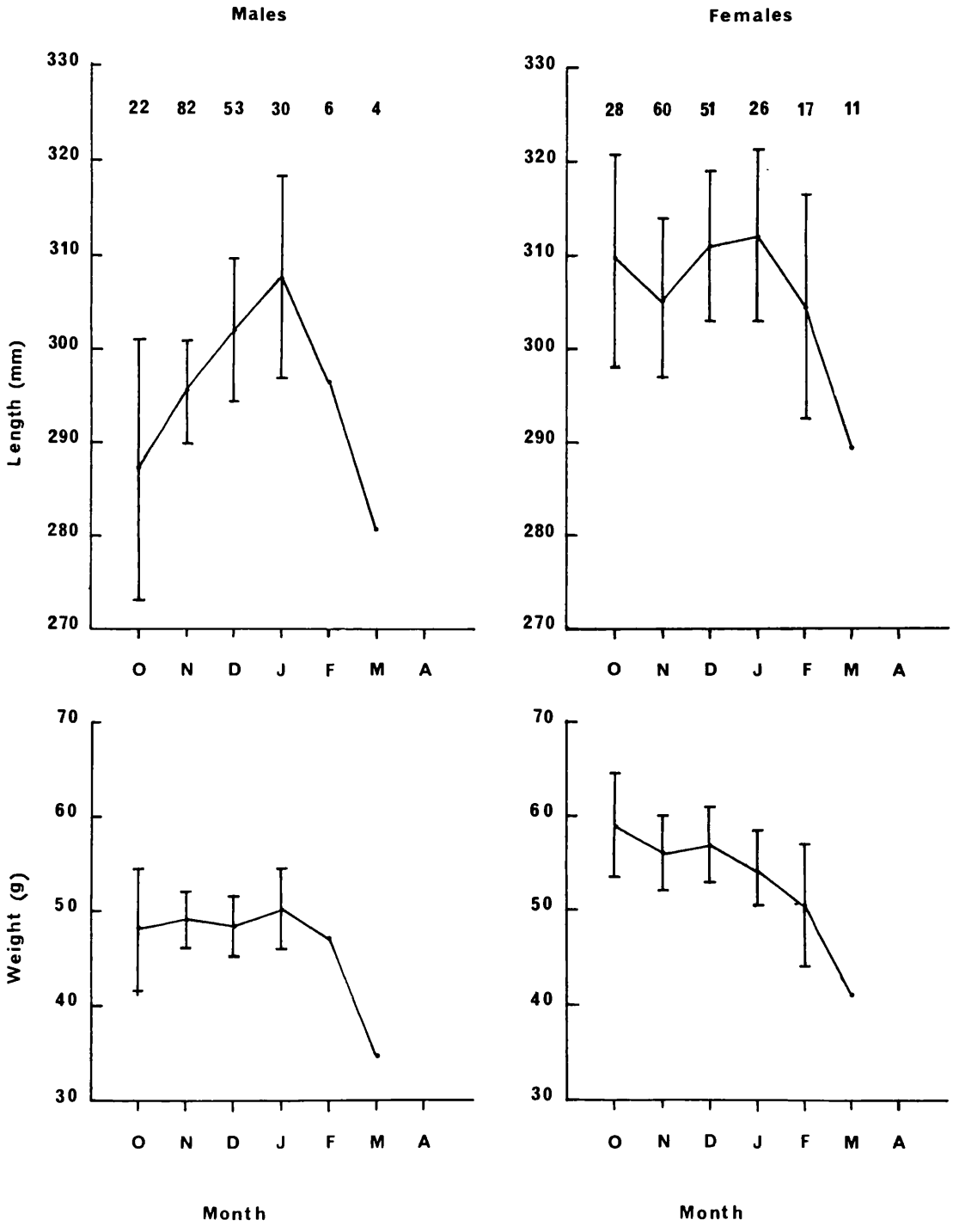
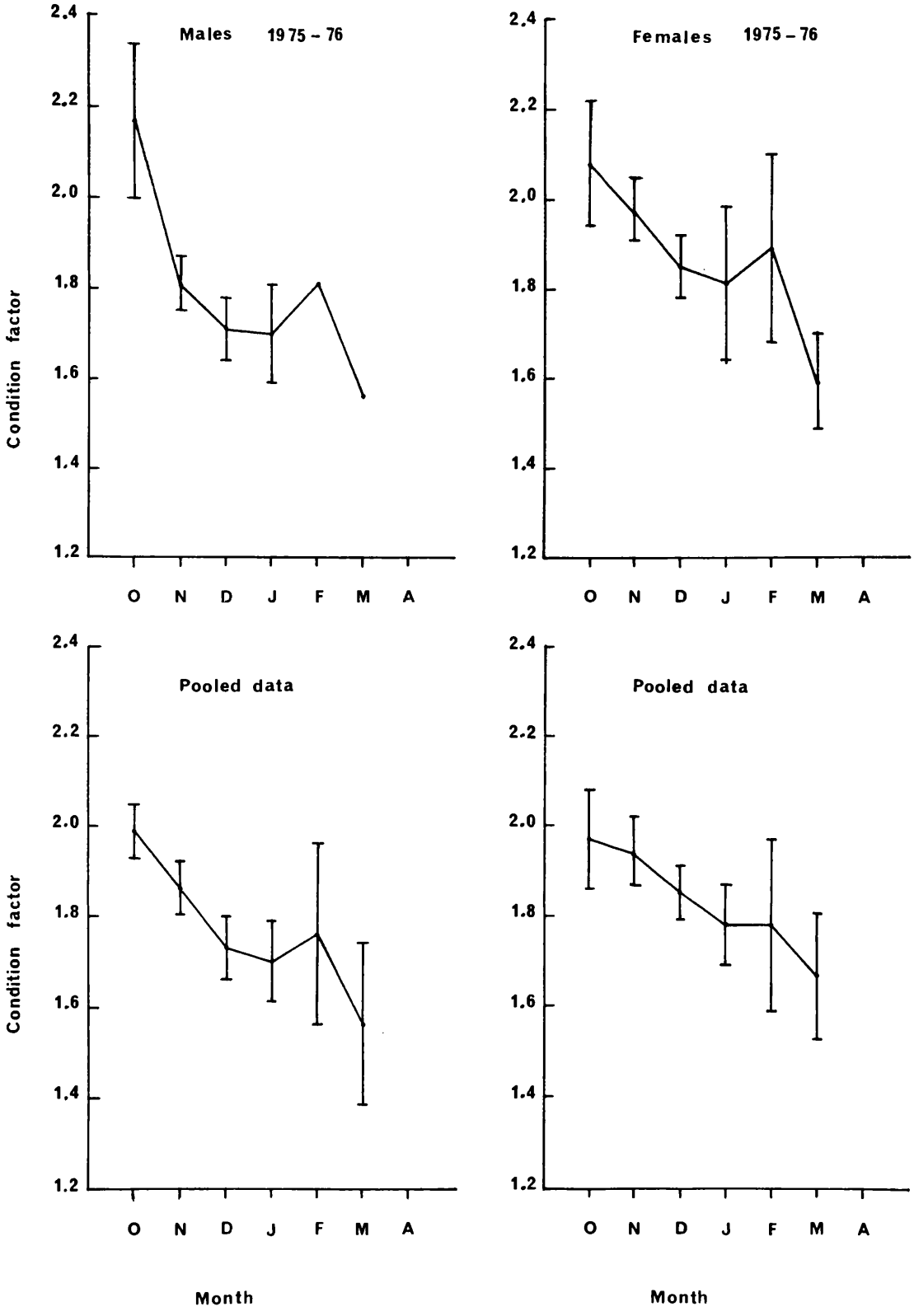

Males
Females
Length (mm)
Weight (g)
330
320
310
300
290
280
270
70
60
50
40
30
22 82 53 30 6 4
28 60 51 26 17 11
O N D J F M A
Month

FIGURE 12

The condition factor for typical forms collected from Oldbury between October 1973 and March 1976.


Males 1975 - 76
Females 1975 - 76
Pooled data
Pooled data
Condition factor
Condition factor
2.4
2.2
2.0
1.8
1.6
1.4
1.2
O N D J F M A
Month
Month

greater than that of the testis ($p < 0.01$). In October, the mean diameter of the ova was 0.546 mm rising to 0.584 in January (Table 4). While some males caught in September contained spermatogonia, all others were characterised by the possession of primary spermatocytes (Plate 3a).

Although the relative weight of the gut was invariably greater in October than in either November or December, there was no consistent pattern of change between the samples representing the last two months (Appendix 1-8, Figure 13). Thus, the mean gut ratio for the three successive months in 1974 was 0.87, 0.60 and 0.53% in females and 0.87, 0.47 and 0.53% in males. These data are consistent with the calculations for the gut diameter ratio which in both males and females was greater in October than in December (Figure 14).

Despite the decline in diameter the gut showed a similar histological picture in December to that observed in October (Plate 3c,d). In other words, although there had been some regression in size, well developed longitudinal folds were still present and had not started to show clearly the characteristic massive degeneration that occurs in fresh water (Larsen, 1973).

A significant difference ($p < 0.05$) was found in the hepatosomic ratio of the two sexes in October, November and December and during this period the values for the females liver were greater than those for the males (Appendix 3-8). No consistent trend of increase or decrease was observed over this period.

Little variation was found between the mean values for the disc although it might be biologically significant that the values for both males and females were greater in spent animals than in any of the estuarine animals (Figure 15). It is also noteworthy that the lowest

TABLE 4

The ova diameter (±95% confidence limits) for typical and praecox forms of the lamprey collected at Oldbury between October and March and also for praecox forms held in the laboratory until May when they developed secondary sexual characters

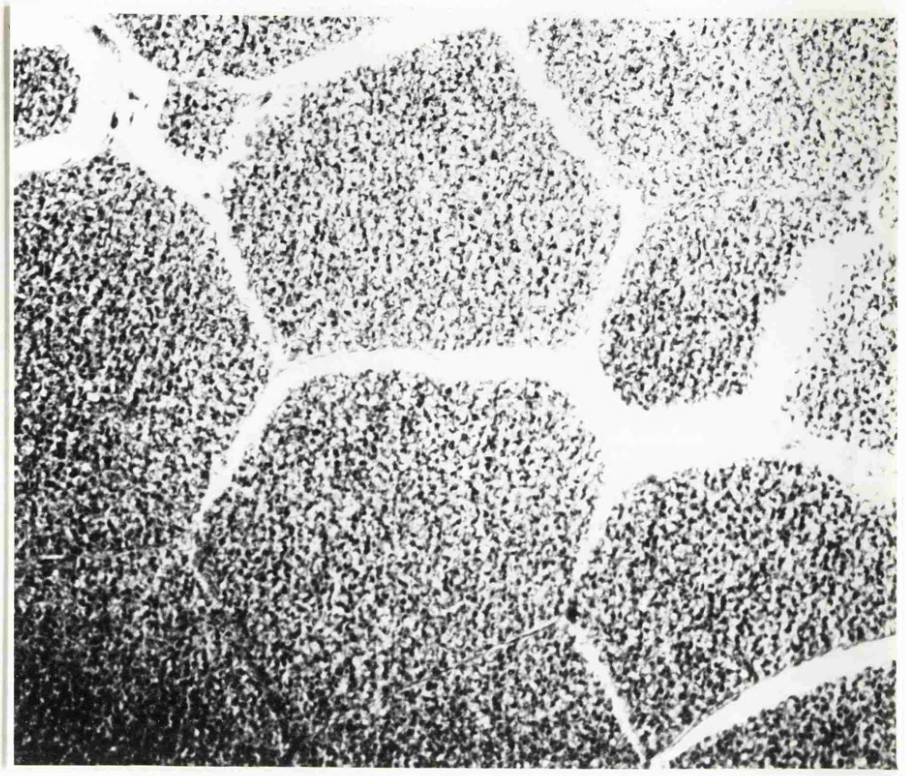
MONTH	OVA DIAMETER	LENGTH OF ANIMALS	WEIGHT OF ANIMALS	NUMBER OF ANIMALS
October	0.546 ± 0.013	355.6 ± 0.89	79.97 ± 5.58	9
November	0.547 ± 0.12	296.5 ± 1.82	48.75 ± 8.23	12
December	0.578 ± 0.015	339.7 ± 2.34	73.16 ± 4.08	8
January	0.584 ± 0.007	321.5 ± 1.75	60.85 ± 8.75	6
February	0.545 ± 0.007	251.0 ± 1.802	26.05 ± 6.05	5
March	0.576 ± 0.023	248.0 ± 3.902	26.13 ± 2.262	4
May	0.866 ± 0.054	213.1 ± 3.42	24.60 ± 4.82	7

PLATE 3a

Testis of a typical form *Lampetra fluviatilis*.

PLATE 3b

Testis of a praecox form *Lampetra fluviatilis* obtained in March.



3a

3b

FIGURE 13

The mean gut and gonadosomic ratio (±95% confidence limits) of typical forms collected from

Oldbury •

Tewkesbury ■

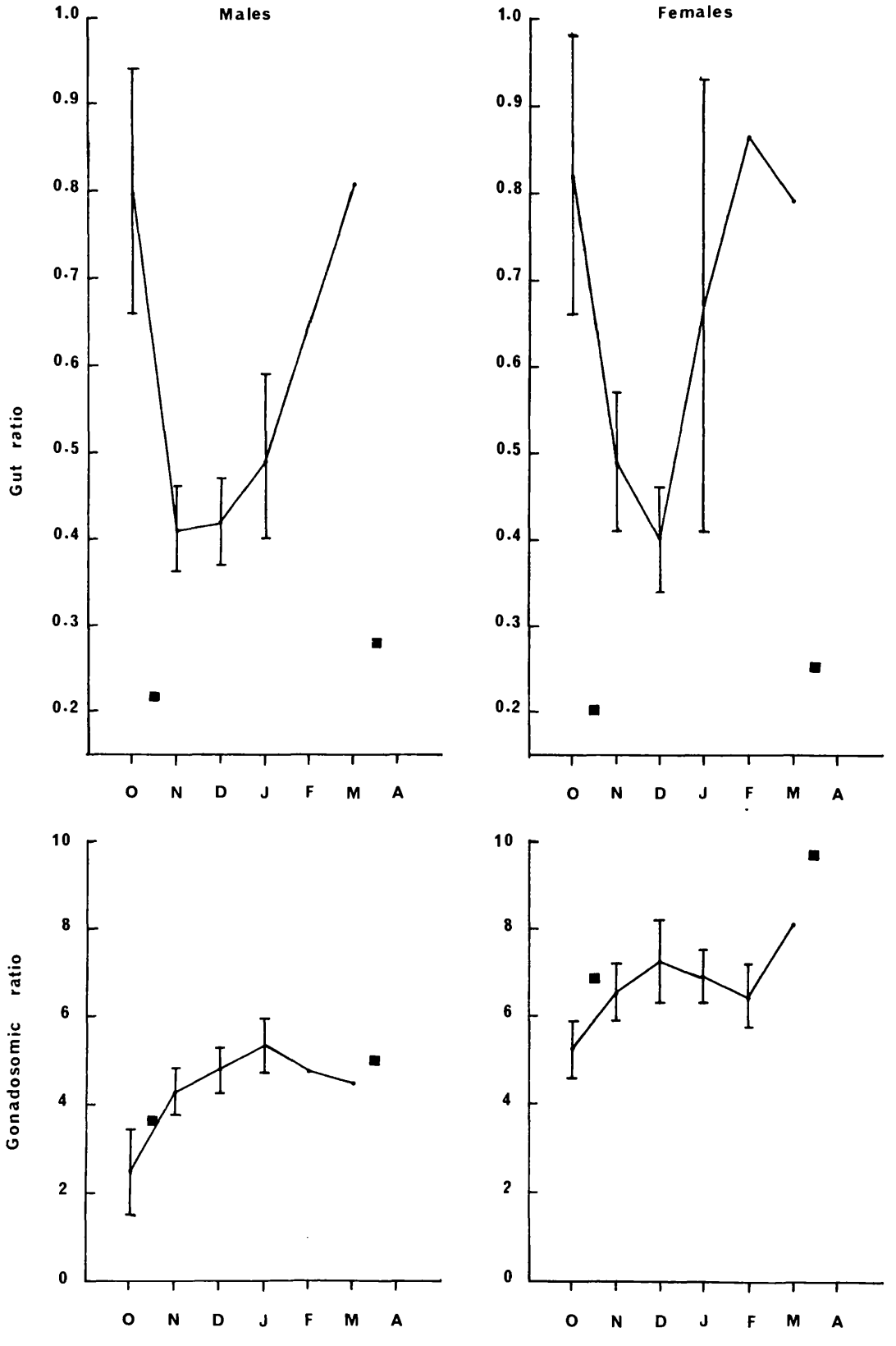

Males
Females
Gut ratio
Gonadosomic ratio
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
10
8
6
4
2
0
O N D J F M A

FIGURE 14

The mean diameter of the gut expressed as a percentage of the total length, together with the 95% confidence limits.

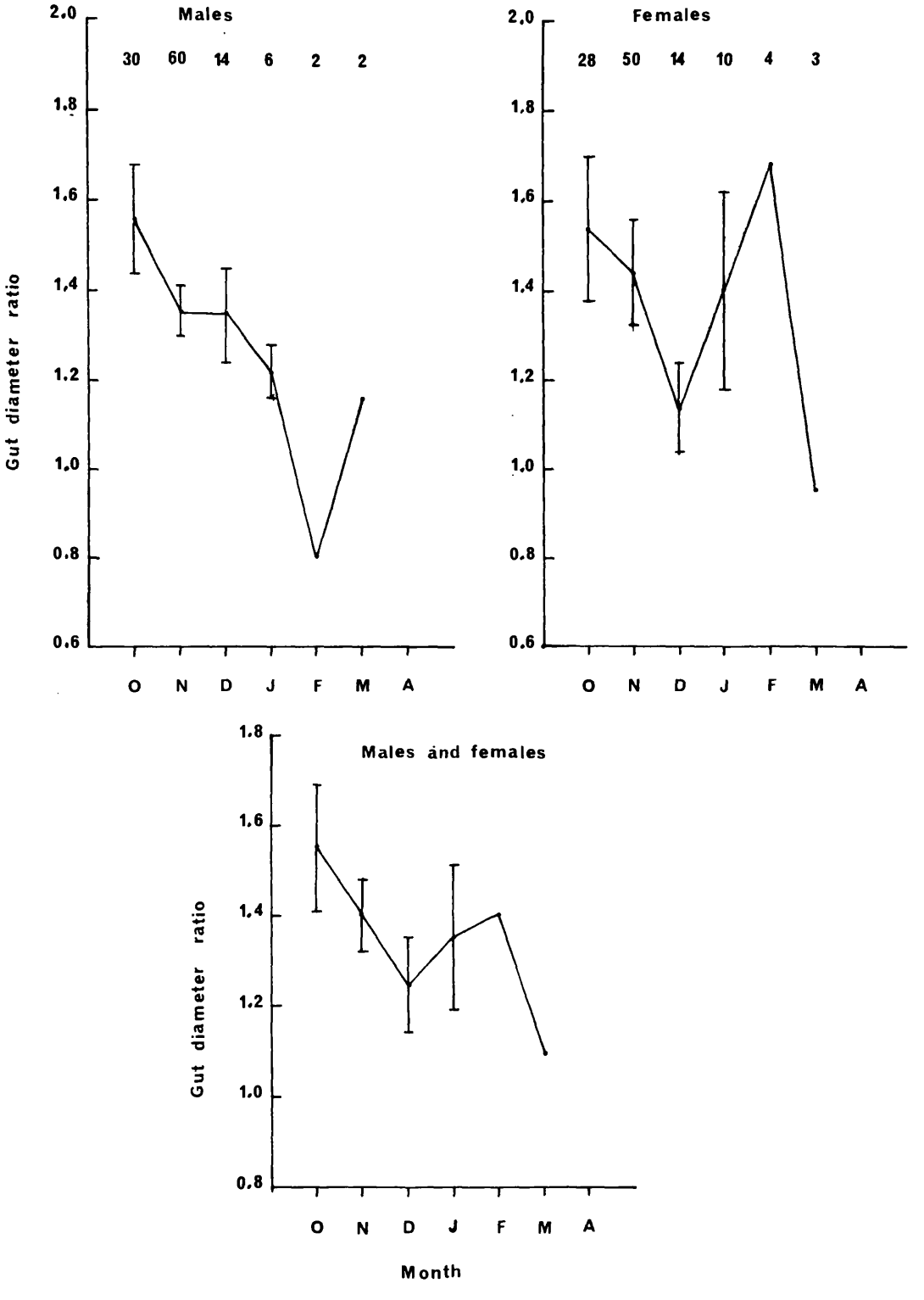
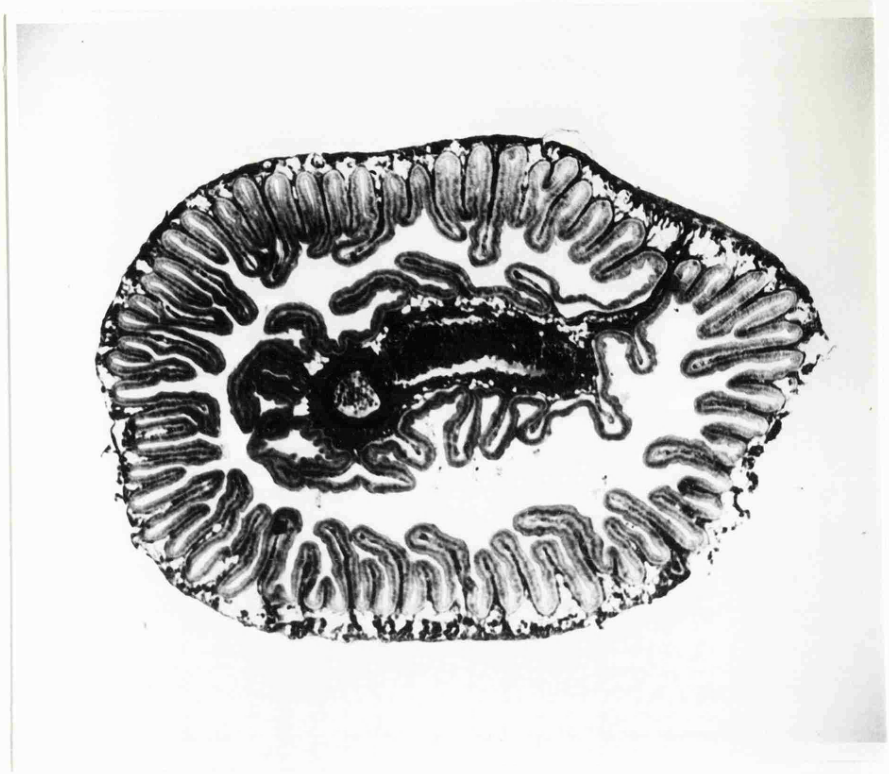

Males
30 60 14 6 2 2
Females
28 50 14 10 4 3
Males and females
Gut diameter ratio
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
O N D J F M A
Month

PLATE 3c

Cross section of the gut of typical form *Lampetra fluviatilis* obtained in October in the middle reaches of the Severn Estuary.

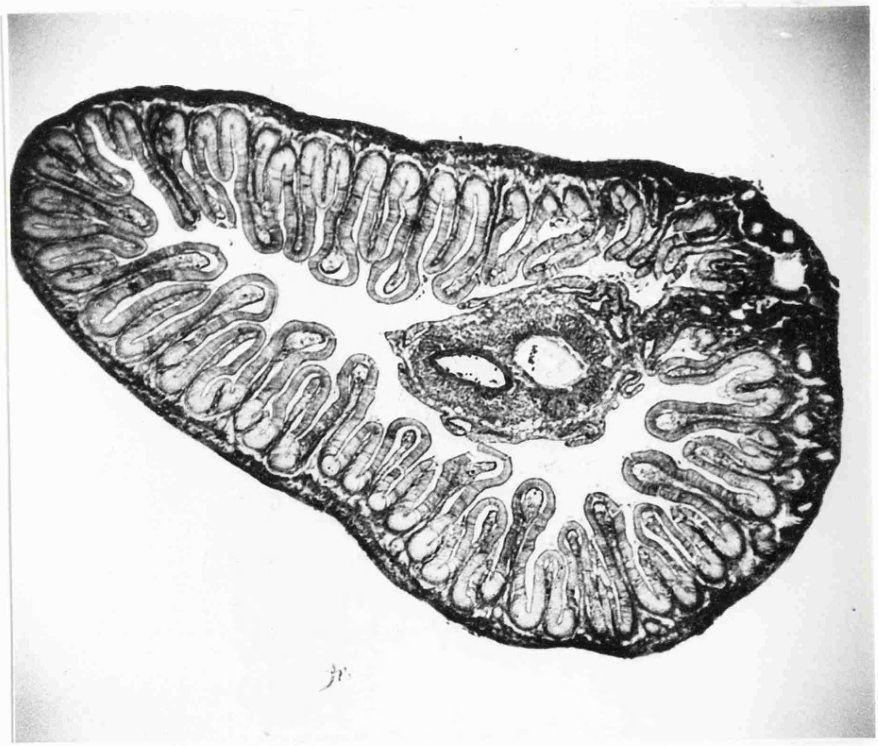
PLATE 3d

Cross section of the gut of typical form *Lampetra fluviatilis* caught in December.



3c

0 µm 50



3d

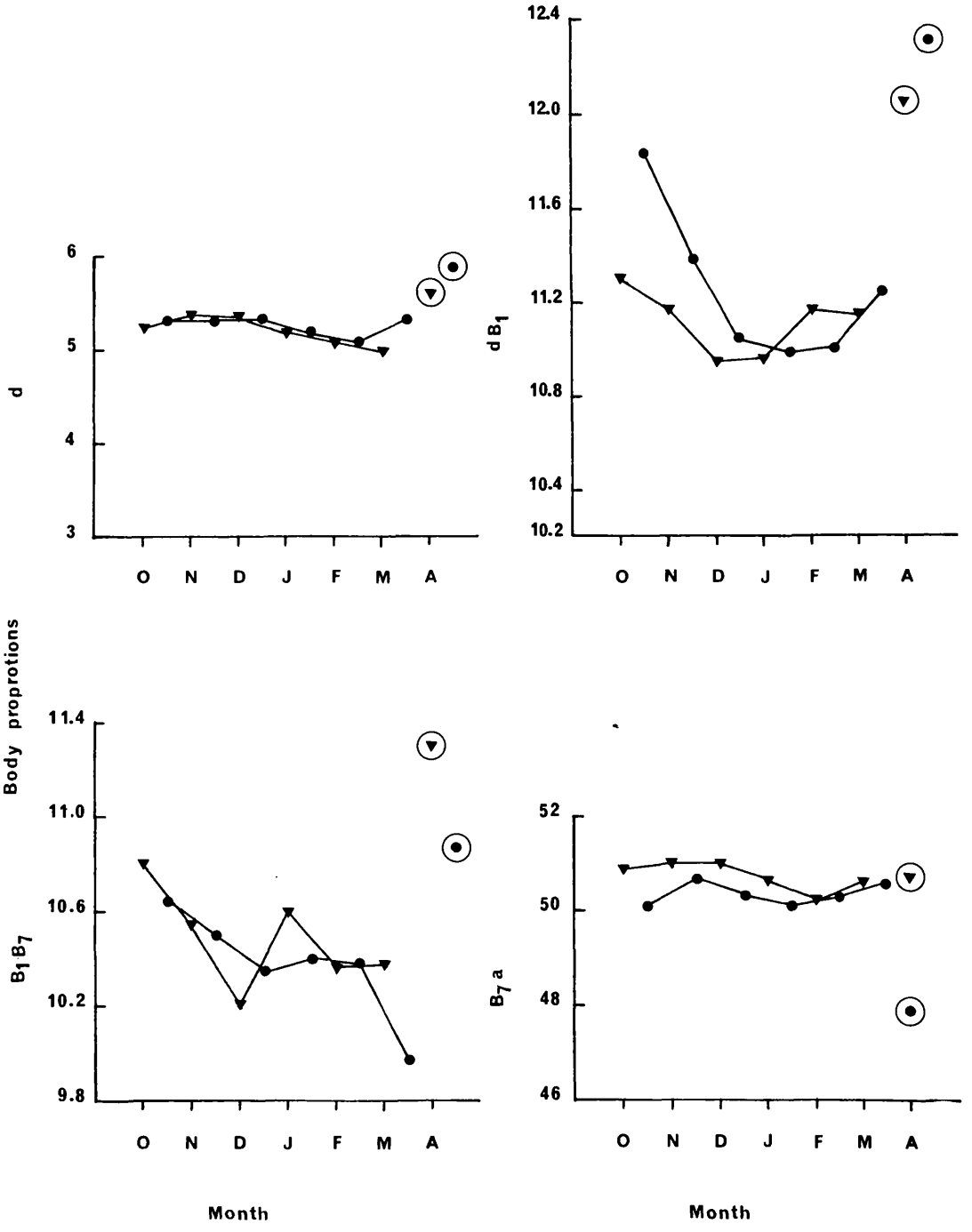
FIGURE 15

The mean length of the disc, prebranchial, branchial and trunk regions, each expressed as a percentage of the total length

Males ●

Females ▼

Tenbury ◯


Body proprotions
d
6
5
4
3
O N D J F M A
dB1
12.4
12.0
11.6
11.2
10.8
10.4
10.2
O N D J F M A
B1B7
11.4
11.0
10.6
10.2
9.8
O N D J F M A
Month
B7a
52
50
48
46
O N D J F M A
Month

means were recorded after December. The change of pattern in the prebranchial region is however more marked. Thus, after an initial decline it increased precipitously after spawning.

The branchial region in both sexes declined consistently between October and December before fluctuating markedly (Figure 15). After spawning the values rose again, a feature no doubt attributable to the well developed branchial skeleton would help this region to maintain its length during the overall shrinkage that occurs during the spawning migration.

The trunk region of the females was consistently greater than that of males between October and January when samples were large. Although its relative length did not differ further in females it declined appreciably in males. Almost the converse pattern occurred in the tail with the relative length declining in females and increasing in males. This suggests that the female utilises reserves and body tissues in the tail more than is the case in the male. That the female may require a great degree of mobilisable material is indicated by the much larger size of the gonad it develops. In mature animals this is reflected by gonadosomic ratio of approximately 16% in females compared with one of 6% in males (Larsen, 1973). This situation just described for *L. fluviatilis* parallels that already described for *L. planeri* (Bird, 1976).

The height of the first and second dorsal fins increase markedly between the early migrants and the situation found after spawning at which time the fin height of the males is higher than that of females (Figure 16). The overall trend seen particularly in the second dorsal fin is broken in February and March. There is also a break in February in the pattern of decline between the two dorsal fins (Figure 16).

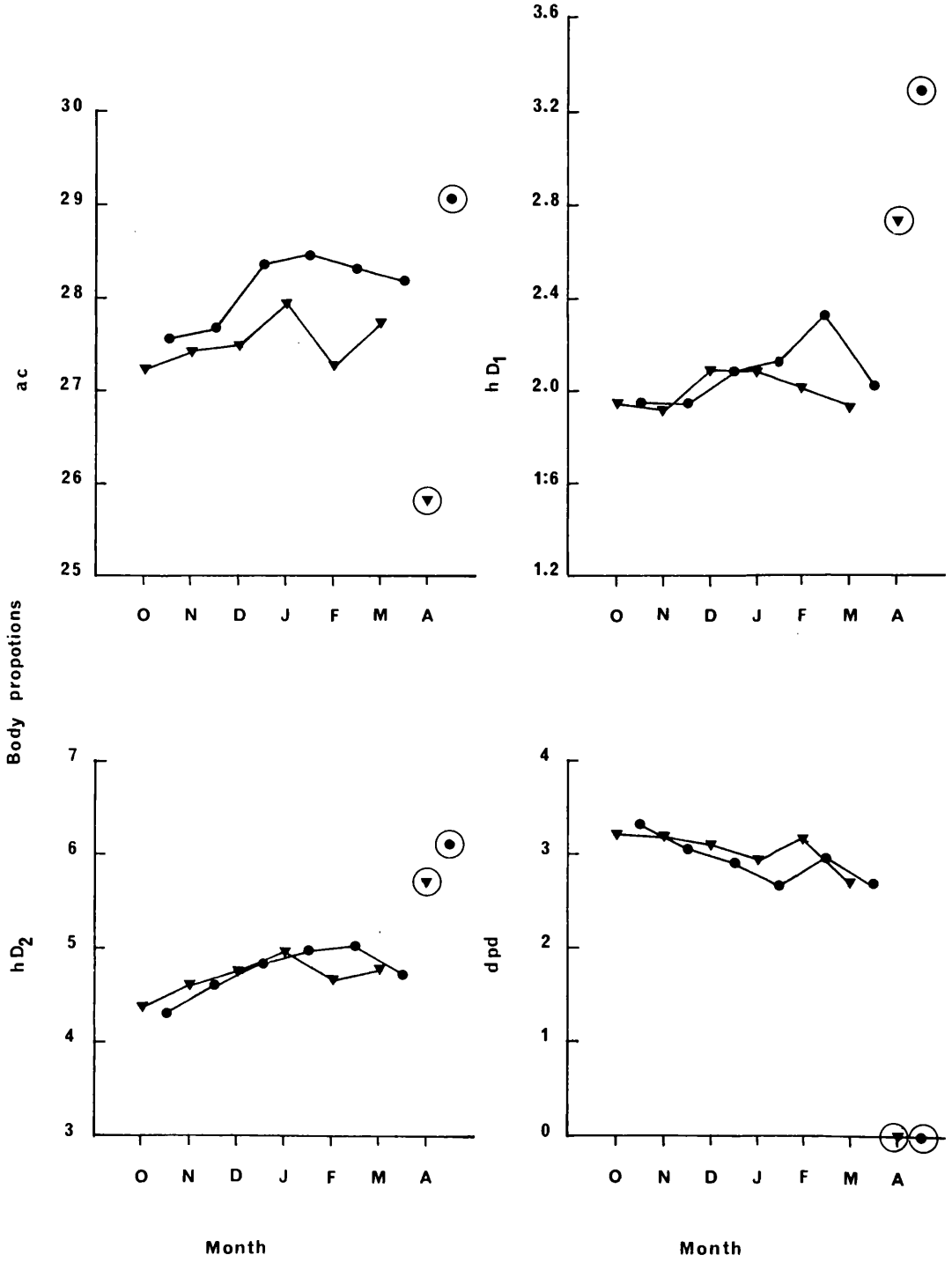
FIGURE 16

The mean length of the tail, height of the first and second dorsal fins and the distance between the two dorsal fins, each expressed as a percentage of the total length

Males ●

Females ▼

Tenbury ◯


Body propotions
a c
30
29
28
27
26
25
O N D J F M A
h D1
3.6
3.2
2.8
2.4
2.0
1:6
1.2
O N D J F M A
h D2
7
6
5
4
3
O N D J F M A
Month
d pd
4
3
2
1
0
O N D J F M A
Month

The full pooled data for mean lengths, weights, condition factors, gut ratio, gonadosomic ratio, hepatosomic ratio and all body measurements are given in Appendices 9-12.

3.4. CHARACTERISTICS OF THE PRAECOX FORMS

After separating out the praecox forms using the length-frequency curves, they were subjected to the same morphometric analyses as the larger typical forms. Because, however, of their relatively smaller number compared with the typical forms, the data for January, February and March in the different years will not be considered separately. As can be seen from pooled data for the three years (Tables 5 & 6) the mean lengths and weights in each of these three months were similar. Thus, the length ranged from only 233.3 to 241.3 mm and the weight from only 20.4 to 21.9 g. While the lengths and weights for January are based on small numbers of males (10) and females (8), the minimum number used for measurements of both sexes in February and March was 28. It should be noted, however, that the means for other measurements (i.e. gut, gonad and liver) are based in all three months on far smaller numbers. Since in the case of the January collections, the sample size for these measurements was in some cases as few as three, the data for January must be treated with caution. In the much larger samples for the following two months it is almost certainly of significance that, between February and March, the gut ratio declined and the gonadosomic ratio increased. In February and March, the mean oocyte diameters were 0.545 and 0.576 mm respectively (Table 4). Seven animals held in the laboratory at 10°C until May when they reached maturity had a mean oocyte diameter of 0.866 mm.

TABLE 5

The mean values (±95% confidence limits and sample size) for males of the praecox forms collected from the Oldbury Power Station

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER
January	235.3 (10) ±5.654	20.4 (10) ±3.18	4.57 (10) ±0.69	0.64 (10) ±0.21	1.36 ±0.18
February	238.4 (33) ±6.53	21.7 (33) ±1.73	3.93 (22) ±0.33	0.86 (22) ±0.22	1.19 (22) ±0.062
March	236.2 (28) ±5.73	20.04 (28) ±1.5	4.82 (13) ±0.73	0.58 (13) ±0.11	1.31 (13) ±0.24

TABLE 6

The mean values (±95% confidence limits and sample size) for females of the praecox forms collected from the Oldbury Power Station

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER
January	233.3 (8) ±12.79	21.1 (8) ±3.100	8.18 (3) ±1.18	0.51 (3) ±0.073	1.47 (3) ±1.400
February	241.3 (30) ±5.51	23.6 (30) ±1.47	6.89 (22) ±0.71	0.68 (22) ±0.11	2.02 (22) ±0.15
March	236.4 (27) ±8.37	21.9 (27) ±2.047	8.36 (12) ±1.068	0.54 (12) ±0.12	1.90 (12) ±0.16

The fecundity of these animals was 1,314 ranging from 1,100 to 1,448. That the gonadosomic and oocyte diameter of these animals is much greater than in the typical forms caught at Oldbury (Figure 13, Table 4) suggests that there is little doubt that the praecox forms will enter and spawn in the rivers at some time in the spring or summer. In this context, it is worth noting that spermatozoa were present in one animal sampled in mid March although this was not typical since in all other cases the testis contained only primary spermatocytes. At the same time the well developed guts found in the praecox forms (Plate 3b) suggest that feeding has only recently been completed. As with October typical forms, the gut possessed well developed longitudinal folds and there were no marked signs of degeneration (Plate 3f).

In the months of peak abundance, January to March, the ratio of males to females in pooled data for the different years ranged from 1:0.76 to 1:1.72,with the overall mean for all animals being 1:1.06.

3.5. TYPICAL FORMS WITH UNUSUAL CHARACTERISTICS

The picture which emerges from the Severn is one of a population in which there are two main groups. Thus, there is firstly the typical forms with the characteristics exhibited in the period of their peak abundance, i.e. between October and either December or January depending on the year and secondly the praecox forms found predominantly between January and March. However, the very large guts in some of the small number of larger animals from February and March suggest that there is a third group which do not fall into either of the above categories. For example, the relative weight and diameter of the gut in some of the animals were greater than those found in any

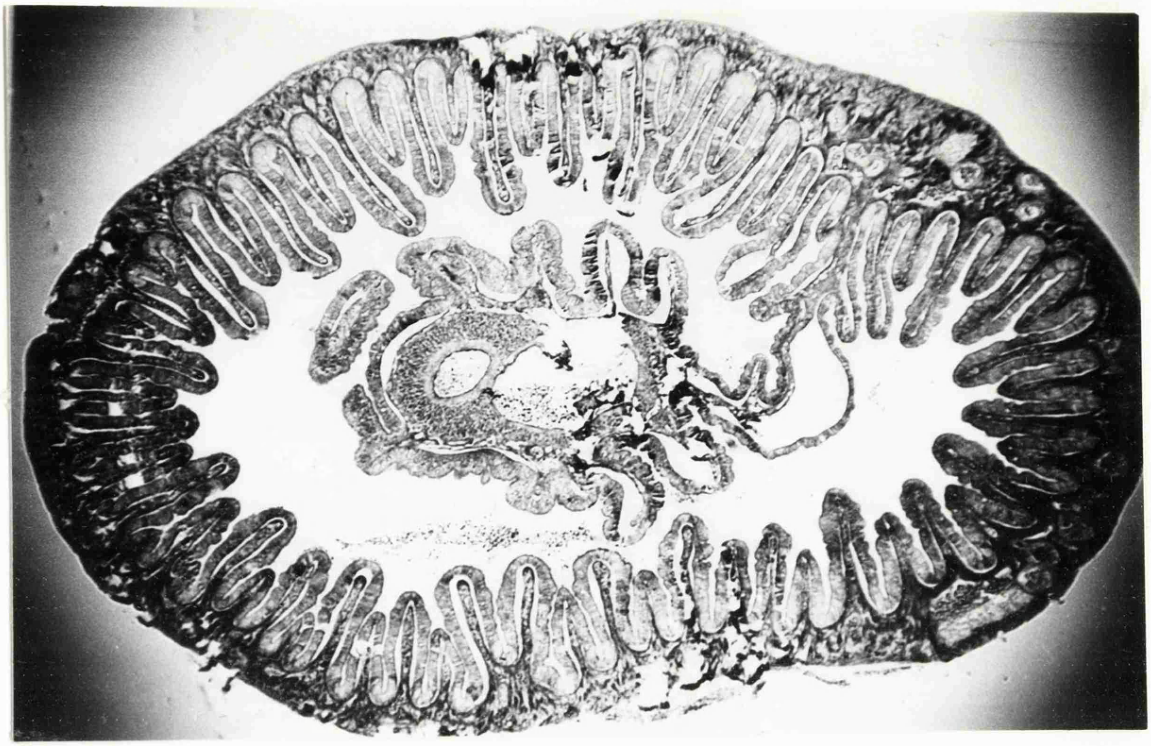
PLATE 3e

Cross section of the gut of typical form *Lampetra fluviatilis* caught in March, showing a larger gut and more well developed folds than those obtained in October or December.

PLATE 3f

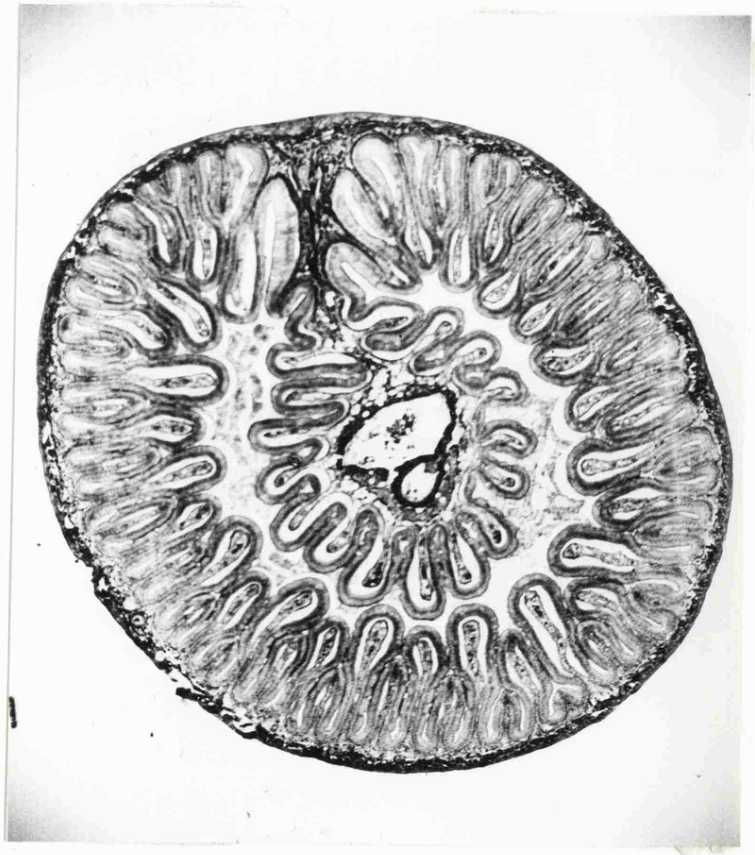
Cross section of the gut of a praecox form *Lampetra fluviatilis* obtained in January, showing a well developed gut.

3e



0 µm 50

3f



of the migrants passing through the estuary in October. Furthermore, the degree of development could also be seen to be greater at the histological level. Thus, the surrounding epithelium was larger and contained many small blood vessels and the lumen was clearly of greater diameter (Plate 3e).

Evidence for the increasing influx of animals of the typical large size, but with different characteristics is also borne out by the fact that although the gonadosomic ratio for females showed a consistent increase between October and December, slightly lower values were obtained in January and February (Figure 13). In fact, the gonadosomic ratio of females was lower than in the first catches made in fresh water at Tewkesbury in October. At the same time, they were still greater than at Oldbury in October, and the March values were the highest recorded at any time from Oldbury. The gonadosomic ratio of males increased until January before undergoing a slight decline in February and March. This trend in males should not be taken, however, as necessarily indicating a less mature testis since this organ is known to lose weight at this stage through a loss of water (Larsen, 1973). The testis of all the males of typical size caught between January and March contained only primary spermatocytes, as was the case with all animals caught between October and December.

The pattern of change in gut ratios and in the gonadosomic ratio for females after December supports the view made above that the animals of typical size contain representatives with rather different characters. Further support comes from the great variation that is found in the animals of typical size. For example, the gut ratio in the females from February ranged from as low as 0.24 to as high as 1.44 and in the females the gonadosomic ratio ranged from 4.42 to 8.01.

On the basis of these data it is proposed that animals with low gut and high gonadosomic ratios represent the tail end of the typical forms found in large numbers during the autumn and early winter. Contrasting with this group are the animals with large guts and low gonadosomic ratios that represent a very much smaller number of animals that do not start entering the estuary until the period between January and April.

3.6. HAEMATOCRIT AND HAEMOGLOBIN CONCENTRATION

The mean values for the haematocrit and haemoglobin concentration did not show any conspicuous downward trend between November and December (Table 7). This presumably reflects the fact that the haemopoietic tissue in the fat column is still active at this time (Percy and Potter, 1976). The much reduced haemoglobin levels in spent animals in April may likewise reflect the degeneration of the fat column during the spawning run as well as the breakdown of the erythrocytes which occurs during the terminal phase of the life cycle (Larsen, 1973).

The range in mean values for the haematocrit of 39.1 - 40.6 obtained for animals in estuarine water at Oldbury is greater than the 36.5 given by Ivanova Berg and Sokolova (1959) for animals caught in fresh water in the River Neva in the autumn. Likewise, the range in haemoglobin concentration of 13.3 - 14.5 g% for the same period is slightly above the mean of 13 given by Ivanova Berg and Sokolova. The reduced values in the latter study compared with those from Oldbury are consistent with the breakdown in the red blood cell

TABLE 7

The haematocrit and haemoglobin concentration of lampreys caught at Oldbury (November to January) and sexually mature praecox forms held in the laboratory (May).

MONTH	SEX	HAEMATOCRIT	HAEMOGLOBIN g%
November	Male	39.14 ± 3.82 (9)	13.95 (1)
	Female	39.61 ± 3.91 (11)	13.29 (4)
December	Male	40.12 ± 3.98 (16)	14.54 ± 1.27 (17)
	Female	40.60 ± 3.403 (17)	13.89 ± 2.13 (5)
January	Male	37.97 ± 3.044 (8)	13.02 ± 1.58 (7)
	Female	37.59 ± 5.502 (5)	14.60 (3)
May	Male	-	9.16 (4)
	Female	-	7.18 ± 1.038 (8)

forming ability of the fat column which commences during the upstream migration (Percy and Potter, 1976). By the spring the haematocrit and haemoglobin concentration of the Neva lampreys had declined to 29.0 and 8.8 g%. The latter value is similar to the 7.18 and 9.16 g% recorded in this study for sexually mature female and male praecox forms.

3.7. IONIC COMPOSITION

In 1973/74, the animals brought back from estuarine water at Oldbury were kept for one week in well aerated fresh water before analysis of the ionic composition of their blood. Throughout successive months the levels of the sodium and chloride ions showed a downward trend (Table 8). Thus, sodium declined from a mean value of approximately 150 mM l^{-1} in October to about 110 mM l^{-1} in January. Three female praecox forms caught in March had an even lower mean value of 103 mM l^{-1}. After spawning the mean of four males was 94 mM l^{-1} while a single female yielded a value of 76.5 mM l^{-1}. With respect to chloride, the levels of both sexes declined from about 130 mM l^{-1} in October to about 120 mM l^{-1} in January (Table 8). At spawning, values of 106 and 86 mM l^{-1} were obtained for males and females respectively. Although the potassium levels showed a decline between October and November, the fact that, at least in males, the values in December and January were similar to those of October (3.8 - 4.0 mM l^{-1}) indicate that this ion does not become reduced to the same extent as the other two major monovalent components of the blood. The apparent retention of a similar pattern in potassium in

TABLE 8

Ionic Concentrations (mM l^{-1}) in the plasma of *Lampetra fluviatilis* kept for a week in fresh water. Also included are the number of individual analyses and ±95% confidence limit.

MONTH	SEX	Na mM/l	K mM/l	Ca mM/l	Mg Mm/l	Cl mM/l
Oct 73	Males (5)	149.9 ± 5.14	4.0 ± 0.77	4.0 ± 0.13	0.62 ± 0.08	128.5 ± 1.23
	Females (5)	150.1 ± 4.63	4.6 ± 0.51	4.3 ± 0.08	0.68 ± 0.03	130.8 ± 0.51
Nov 73	Males (9)	139.5 ± 2.46	3.0 ± 0.23	3.9 ± 0.07	0.75 ± 0.22	122.4 ± 1.55
	Females (2)	133.9	2.9	3.85	0.60	121.3
Dec 73	Males (9)	110.5 ± 5.202	3.8 ± 0.68	2.0 ± 0.904	0.84 ± 0.23	122.0 ± 2.46
	Females (6)	102.7 ± 3.92	3.9 ± 0.49	1.8 ± 0.49	0.50 ± 0.24	115.5 ± 2.94
Jan 74	Males (4)	108.1	4.0	1.98	0.50	121.5
	Females (4)	113.1	3.4	2.0	0.38	119.9
Mar 74	Males (-)	-	-	-	-	-
	Females (3)	103.4	2.4	0.3	0.37	120.0
Apr 74 (Spent)	Males (4)	94.1	3.2	0.29	0.40	106.0
	Females	76.5	3.6	0.30	0.30	86.3

spent animals to that of the typical forms caught in January, may, however, represent a terminal increase due to the release of this ion when the red cells break down at the end of the spawning run.

There was also a downward trend in the divalent ions during the period when the animals were in the estuary (Table 8). The pattern was not quite as clear cut, however, in a number of cases. For example, the magnesium values for males remained between 0.5 and 0.84 mM l^{-1}. At the same time, evidence for a drop in concentration was found in the calcium of both sexes and in the magnesium of females. The values for divalent ions of the three female praecox forms was in every case lower than that of typical forms caught in October.

In order to ascertain the degree to which animals at Oldbury were able to regulate their ions in an estuarine environment, lampreys caught in November were placed in 100, 70 and 50% sea water. Although seventy percent of the lampreys survived for three days in full strength sea water, they had apparently become so dehydrated that it was impossible to extract their blood by heart puncture. All animals in 70 and 50% sea water survived and analysis of their blood after one week revealed no significant difference between ions in the two groups. Since the various ions are being maintained at a level lower than that of the environment (Table 9), the animals are capable of osmoregulating in 50% and 70% sea water. The response to being placed in full strength sea water suggest however, that they would be unable at this stage to return to sea.

In view of the paucity of data on the ionic composition and osmotic pressure of lampreys in estuarine environments, animals were held for a week in water in which they had been captured in order to avoid any short term fluctuations, these results indicate that the

TABLE 9

The concentration of various ions ($mM\ l^{-1}$) of animals caught at Oldbury in November and transferred for one week to fresh water and 50 and 70% sea water.

HABITAT	Na	K	Ca	Mg	Cl	P mg 100 ml^{-1}
F.W. (26)	121.7 ± 3.83	3.4 ± 0.46	2.9 ± 0.54	0.67 ± 0.21	120.3 ± 2.19	-
50% S.W. (9)	133.6 ± 6.12	3.47 ± 1.19	2.98 ± 0.61	1.17 ± 0.34	125.40± 5.49	3.55 ± 0.24
*50% S.W.	134.8	4.2	2.6	2.2	127.4	-
70% S.W. (8)	137.5 ± 5.32	3.80 ± 0.81	3.18 ± 0.61	1.53 ± 0.609	130.76± 5.071	4.54 ± 1.098

* Values given by Pickering and Dockray (1972)

male and female River lampreys maintain a lower plasma osmotic pressure than the environment, thus reflecting a highly efficient osmoregulatory mechanism (Table 10). Another significant but unexplained fact that emerges is that the osmotic pressure dropped significantly from a mean of 313.0 mosmoles in November to 299.0 mosmoles by February. The implications of this are not clearly understood but the corresponding drop in salinity levels over the same period from 24.5‰ (November) to 20.2‰ might be the possible explanation. Plasma, sodium and chloride values were greatest in the first of these three months. Between November and January, inclusive, however, there was no distinct trend either with time or with salinity (Table 10). Thus, sodium levels ranged between 124.2 mM l^{-1} in females in December 1975 to 138.1 mM l^{-1} in females in December 1974. The lowest chloride values (120 mM l^{-1}) were found in males in November 1974, whereas the highest (137.7 mM l^{-1}) were obtained in November 1975. The constancy of the ion level between November and January suggests that the animals during this period have a similar ability to regulate their ions in estuarine environments.

3.8. IONIC COMPOSITION AND WATER LEVEL OF TISSUES

To ascertain the relative levels of ions in the tissues of males and females in the estuary, animals caught in November were held in the laboratory for one week in a sample of estuarine water from which they had been collected (Table 11). Marked differences were found between the concentrations of ions (measured in mM Kg^{-1} tissue water) of the organs of male and female lampreys. Thus, the means for sodium ranging from 33.2 to 39.1 in the testis were much lower than in

TABLE 10

Ionic concentrations (mM l^{-1}) and osmotic pressure (mosmoles) in the plasma of *Lampetra fluviatilis* kept for one week in estuarine water where salinity values fluctuated. Also included are the number of individual analyses and ±95% confidence limit

MONTH	SEX	Na mM/l	K mM/l	Ca mM/l	Mg mM/l	Cl mM/l	P mg $100ml^{-1}$	OSMOTIC PRESSURE mosm	SALINITY ‰
Oct 74	Males (3)	138.6	3.6	2.6	1.4	132.0	3.9	-	17.0
	Females (6)	140.0 ± 5.63	4.7 ± 0.49	2.6 ± 0.25	1.4 ± 0.25	127.4 ± 5.87	3.8 ± 0.098		
Nov 74	Males (6)	127.3 ± 9.13	3.11 ± 0.99	2.98 ± 0.47	1.38 ± 0.28	120.0	3.76 ± 0.29	-	14.6
	Females (9)	124.7 ± 8.32	3.18 ± 0.93	3.11 ± 0.54	1.51 ± 0.407	125.2 ± 6.401	3.88 ± 0.907		
Nov 75	Males (3)	134.3	4.53	1.80	1.86	137.7	5.13	314.9	24.5
	Females (4)	128.5	3.68	2.45	1.87	131.0	5.01	311.1	
Dec 74	Males (4)	130.9	3.2	2.30	1.18	123.2	3.85		12.8
	Females (5)	138.1 ± 7.74	5.34 ± 0.85	2.36 ± 4.39	1.54 ± 1.21	132.6 ± 8.92	3.82 ± 0.49		
Dec 75	Males (18)	127.96 ± 5.12	3.72 ± 0.58	1.89 ± 0.39	2.09 ± 0.27	127.3 ± 6.18	7.16 ± 2.012	313.4	21.0
	Females (22)	124.20 ± 4.34	3.59 ± 0.71	1.98 ± 0.29	2.16 ± 0.33	127.4 ± 6.35	6.03 ± 1.39	300	
Jan 76	Males (6)	129.5 ±12.69	4.63 ± 1.29	2.00 ± 0.55	2.78 ± 0.36	128.5 ±20.9	7.98 ± 3.03	310.9	17.8
	Females (4)	125.0	3.88	2.05	2.52	126.3 ±11.74	7.34	295	
Jan 75	Males (4)	133.8	4.52	2.21	1.12	126.0	4.05	-	10.0
	Females (4)	128.4	4.21	2.91	1.25	127.5	4.12		
Feb 76	Males (3)	124.2	4.36	1.87	2.43	118.00	6.17	308	20.3
	Females (4)	126.9	4.33	1.90	2.41	123.75 ± 9.302	4.71	290	
Mar 75	Males (8)	127.12 ± 1.61	4.30 ± 0.89	2.43 ± 0.12	1.21 ± 0.23	117.32 ± 2.99	4.41 ± 0.23	-	12.4
	Females (5)	126.62 ± 3.59	4.21 ± 0.79	3.32 ± 0.26	1.21 ± 0.103	126.6 ± 3.59	4.71 ± 0.26		

TABLE 11

Ionic concentrations (mM kg^{-1} gonad water) in the gonads of *Lampetra fluviatilis* kept for one week in estuarine water where salinity values fluctuated. Also included are the percentage of tissue water, number of individual analyses and ±95% confidence limit.

MONTH	SEX	Na	K	Ca	Mg	Cl	% OF WATER
Nov 74	Males (6)	25.28 ± 2.86	117.95 ± 3.97	1.96 ± 0.69	4.03 ± 0.69	5.91 ± 1.52	83.67 ± 0.49
	Females (12)	68.2 ± 5.85	85.67 ± 5.88	9.49 ± 1.027	11.59 ± 1.38	23.23 ± 5.69	51.85 ± 1.62
Dec 74	Males (4)	24.07	123.55	1.73	3.70	4.64	84.08
	Females (5)	61.40 ± 8.67	79.24 ± 2.78	8.88 ± 1.52	10.06 ± 2.78	23.31 ± 5.84	51.46 ± 4.22
Nov 75	Males (3)	39.10	121.49	2.97	13.45	6.97	83.44
	Females (4)	81.55	76.71	9.75	25.40	14.13	54.52
Dec 75	Males (15)	33.27 ± 8.700	111.06 ±11.05	2.45 ± 4.62	9.97 ± 2.68	4.90 ± 2.00	84.2 ± 0.51
	Females (17)	66.59 ± 5.032	77.26 ± 4.52	11.54 ± 2.73	22.18 ± 2.75	11.66 ± 2.48	55.16 ± 0.85

the ovary (66.6 - 81.6). Likewise chloride was also lower in the male gonads (4.9 - 7.0) than that of the female (11.7 - 14.1), and a similar pattern was observed with the divalent ions calcium and magnesium. In contrast, to all the above ions, however, the level of potassium was far greater in the testis than the ovary. Furthermore, this latter ion was in a greater concentration in the testis (111.1 - 121.5) than any other ion was in either gonad. Consistent with the much greater lipid content in the ovary than the testis (Moore and Potter, 1976b), was the reverse situation with respect to water. Thus, the testis and ovary had mean water content ranging from 83.4 to 84.2 and 54.5 to 55.2 respectively.

The pattern exhibited by the liver is in many respects the reverse of that found in the gonads (Table 12). Thus, mean sodium levels for males were higher than those of females, the respective means ranging from 58.7 to 71.1 and from 45.7 to 48.7 respectively. Likewise, the water content was consistently greater in the female than male liver, although the difference was not as marked as with the gonads. Potassium tended to be higher in females than males, whereas the reverse pattern was found with calcium. The latter feature is presumably related to the fact that calcium tends to be accumulated in the eggs (Table 11). No consistent difference was found between the magnesium levels in the two sexes.

With respect to ionic levels in the muscle, no marked difference was found between the sexes (Table 13). Both sodium and chloride levels showed a close correspondence between different months and years, with the means for the sodium ranging from 29.5 to 30.2 mM kg^{-1} muscle water, while that for chloride lay between 16.6 and 19.2 mM kg^{-1} muscle water. Potassium levels were again very high with

TABLE 12

Ionic concentrations (mM kg^{-1} liver water) in the liver of *Lampetra fluviatilis* kept for one week in estuarine water where salinity values fluctuated. Also included are the percentage of tissue water, number of individual analyses and ±95% confidence limit.

MONTH	SEX	Na	K	Ca	Mg	Cl	% OF WATER
Nov 74	Males (6)	69.28 ± 14.97	101.13 ± 15.65	7.73 ± 3.57	11.83 ± 3.59	13.66 ± 4.92	60.15 ± 5.56
	Females (12)	48.77 ± 5.24	122.78 ± 6.29	5.82 ± 1.37	15.19 ± 0.69	18.49 ± 9.78	63.43 ± 3.35
Nov 75	Males (3)	62.75	110.3	6.47	13.37	4.87	59.1
	Females (4)	45.67	113.79	4.75	12.73	17.15	69.21
Dec 74	Males (4)	71.13	108.68	9.25	13.55	8.62	64.18
	Females (5)	48.22 ± 6.86	120.66 ± 7.69	4.80 ± 1.26	13.82 ± 2.108	17.63 ± 4.27	67.33 ± 3.73
Dec 75	Males (16)	58.67 ± 7.73	94.99 ± 6.46	8.25 ± 4.15	13.20 ± 2.42	7.21 ± 1.27	62.76 ± 2.31
	Females (17)	47.69 ± 4.62	98.77 ± 7.15	5.11 ± 3.97	11.54 ± 2.11	17.21 ± 1.04	66.89 ± 2.51

TABLE 13

Ionic concentrations (mM kg^{-1} muscle water) in the muscle of *Lampetra fluviatilis* kept for one week in estuarine water where salinity values fluctuated. Also included are the percentage of tissue water, number of individual analyses and ±95% confidence limit.

MONTH	SEX	Na	K	Ca	Mg	Cl	% OF WATER
Nov 74	Males (6)	29.67 ± 3.108	132.12 ± 4.82	2.87 ± 0.37	12.45 ± 0.73	17.70 ± 2.72	63.15 ± 2.13
	Females (12)	29.58 ± 3.14	135.88 ± 2.83	3.28 ± 1.33	13.77 ± 2.005	16.21 ± 0.305	59.23 ± 2.506
Nov 75	Males (3)	28.7	129.93	4.23	12.20	19.2	65.85
	Females (4)	29.5	128.98	4.38	13.00	18.91	64.05
Dec 74	Males (4)	30.00	133.02	3.81	15.15	17.0	65.35
	Females (5)	30.18 ± 10.104	135.92 ± 6.505	2.92 ± 0.98	13.56 ± 0.41	16.46 ± 0.85	58.80 ± 2.65
Dec 75	Males (12)	29.61 ± 3.53	130.01 ± 7.77	5.70 ± 1.11	11.76 ± 0.86	17.8 ± 0.52	65.07 ± 1.59
	Females (17)	29.8 ± 1.23	129.02 ± 4.56	4.94 ± 1.51	11.06 ± 1.03	18.2 ± 0.25	64.18 ± 1.35

minimum and maximum mean values of 129.0 and 135.9 mM kg^{-1} muscle water. The amount of water in the muscle ranged from 58.8 to 65.9%.

As with muscle, the ion levels in the whole animal between November and January did not differ conspicuously with sex (Table 14). Thus, sodium lay between 50 and 60 mM kg^{-1} body water and chloride between 76 and 95 mM kg^{-1} body water. Potassium was present in the greatest concentration, reaching levels of 99.5 mM kg^{-1} body water. The amount of body water ranged from 61 to 69%.

When the ions are expressed as mM kg^{-1} water, the muscle/plasma ratios for sodium and potassium are 0.056 and 33.23 respectively (Table 15). In other words, the sodium is much more predominant in the extracellular space, whereas the potassium is much more abundant in the intracellular space.

The muscle/plasma ratios for lampreys caught at Oldbury do not differ greatly from those that have been recorded for the Perch by Lutz (1972). For example, the above values for sodium and potassium are comparable with Lutz's ratios of 0.040 and 39.5. With calcium and magnesium, lampreys gave ratios of 1.63 and 7.03, whereas in the Perch the ratio for the former ion was rather lower (0.54) while for the latter it was slightly higher (0.6).

The mean values (±95%) for the inulin space of estuarine, fresh water migratory and spent River lampreys were 27.1 ± 2.1%, 26.0 ± 1.93% and 20 ± 2.015%. The only other values for inulin space are those of Bull and Morris (1967) who recorded a mean of 24.4 for ammocoetes of *Lampetra planeri*.

TABLE 14

Ionic concentrations (mM kg^{-1} body water) in the whole body of *Lampetra fluviatilis* kept for one week in estuarine water where salinity values fluctuated. Also included are the percentage of body water, number of individual analyses and ±95% confidence limit

MONTH	SEX	Na	K	Ca	Mg	Cl	% OF WATER
Nov 74	Males (6)	58.20 ± 3.44	99.51 ± 9.47	2.61 ± 4.303	4.50 ± 2.58	94.95 ± 2.75	61.60 ± 3.22
	Females (3)	50.63	89.21	2.52	6.13	63.81	61.03
Dec 74	Males						
	Females (4)	59.12	84.62	3.50	6.22	76.83	61.20
Dec 75	Males (6)	49.95 ± 2.99	87.77 ± 4.012	1.08 ± 4.013	4.01 ± 0.203	85.26 ± 20.41	68.31 ± 4.23
	Females (5)	52.84 ± 8.36	84.78 ± 6.63	1.20 ± 0.45	4.33 ± 0.46	84.00 ± 27.34	68.76 ± 5.22
Jan 76	Males (5)	52.72 ± 5.98	93.48 ± 11.34	1.46 ± 0.072	4.96 ± 0.51	94.69 ± 23.29	66.56 ± 1.59
	Females (5)	53.08 ± 12.37	86.5 ± 30.56	1.48 ± 0.45	4.59 ± 1.602	90.25 ± 38.93	67.53 ± 4.75

TABLE 15

A comparison between extra- and intracellular cation concentration in River lampreys (mM / Kg water)

	Na	K	Ca	Mg	TOTAL
[1]Muscle* N = 26	7.52 ± 0.48	142.56 ± 3.32	4.31 ± 0.69	13.07 ± 0.44	167.47
[2]Plasma**	133.79	4.32	2.64	1.86	142.61
$\frac{\text{Muscle}}{\text{Plasma}}$	0.056	33.23	1.63	7.03	1.17

[1] mM/kg Cell Water

[2] mM/kg Plasma Water calculated from Table 10.

* Calculated from this formula (Lutz 1972)

$$Cc = \frac{\frac{Cm - Cp\ clsp}{100}}{1 - \frac{clsp}{100}}$$

Cc = intracellular concentration mM/kg cell water

Cp = Plasma ionic concentration mM/kg plasma water

Clsp = Chloride space

4. DISCUSSION

The samples collected from the Power Station at Oldbury have revealed a number of important aspects regarding the migration of the River lamprey into the Severn Estuary. There can be little doubt, for example that water discharge is an important factor initiating the movement of River lampreys into the Severn Estuary from the marine environment of the Bristol Channel.
This point is well illustrated by the very late onset of the migration in the autumn of 1972, a period when there was little rain and its far earlier inception in the much wetter autumn of 1976. Furthermore, in the season of 1975/76, when by far the greatest numbers of lampreys were caught, a marked increase in the size of the 24 h samples occurred on four separate occasions just after there had been a rise in water discharge. The influence of water discharge on the migration from the sea to the estuary is consistent with the observations that this environmental factor is also of importance in bringing about movement once the animals have entered the rivers (Seligo, 1926; Buchholtz, 1938). There have, however, been suggestions that the data on which the latter correlations have been based might be misleading since catches were generally made at weirs and dams over which animals would tend to move only when water levels rose (Lanzing, 1959; Hardisty and Potter, 1971b). Since the sampling at Oldbury is not based on such a barrier effect, this argument would not appear to be valid, at least with respect to movement within the estuary.

Although Applegate (1950) recognised that an increase in numbers of migratory landlocked Sea lampreys was associated with increased water discharge, he suggested that a more important factor

was the drop in temperature that this produced. In view however, of the fact that, irrespective of other conditions, the first influx of animals occurred in each year at about 14°C, the influence of temperature should not be discounted. Yet, since no consistent and clear correlation could be demonstrated between the pattern of change in temperature and water discharge throughout the whole of the long sampling period, it would appear unlikely that a drop in temperature had a major influence on the initiation of the movement of *L. fluviatilis*. Furthermore, there is one major difference that should be noted between the migration of the River and Sea lampreys. In the former species the migratory period extends from as early as July to the following March or April whereas in the latter the migration is of shorter duration and does not commence until the spring or early summer (Hardisty and Potter, 1971b). Thus, the relationship between the effects of water discharge on temperature will vary at different times of the year far more in areas where potential migratory *L. fluviatilis* are found than will be the case in *P. marinus*.

The absence of any clear correlation between lunar periodicity and the movement of upstream migrating lampreys would appear to be at variance with the finding of other workers who observed that the numbers caught in rivers were greatest on moonless nights (Seligo, 1926; Buchholtz, 1938; Ryapolova, 1964). This appearent anomaly may be attributable, however, to the fact that the Severn estuary carries an exceptionally high level of particulate matter (Boyden & Little, 1975) which must considerably reduce light penetration. In view of the numerous references to catches being greatest at night, and to the avoidance of light by lampreys during darkness (Abakumov,

1956), it is astonishing to note that Tesch (1967) should have found that maximum catches of River lampreys in the River Elbe were made near the period when a full moon was present.

The data collected from the Power Station at Oldbury has also shown that the population in each spawning run period consists predominantly of two different size groups which represent what Berg (1948) has called typical and praecox forms, these having body lengths of approximately 300 and 230 mm respectively. While the typical form is generally found in considerable numbers in collections prior to January, the reverse situation pertains in February, March and April. This is consistent with the situation in the River Neva which flows into the Gulf of Finland where appreciable numbers of the praecox forms were found in the delta of this river in October and November (Ivanova Berg, 1936; Berg, 1948). The mean length of the male and female praecox forms in these two months was given as 227 and 224 mm, which is approximately 10 mm shorter than the praecox forms caught in the Severn. By contrast, the typical forms in the Severn during the autumn were generally at least 10 mm smaller than they were in the Neva where the mean lengths for males and females ranged from 31-32 and 32-34 cm respectively.

The typical forms of the River lamprey caught in the Severn are in fact smaller than those found in most other European rivers. For example, a mean length of 356-408 mm is given for a Lithuanian river by Gaygalas and Matskevichyus (1968) which is similar to the data presented by Lanzing (1959) for the River Meuse. The consistently larger size of River lampreys caught in the Baltic and North Sea compared with those of the River Severn suggests that feeding conditions in the former areas are probably better than off the west coast of England.

Another important finding was that animals of typical size enter the estuary over a very long period. Thus, although they can occasionally be present in relatively very low numbers as early as July or August, they can be taken in the estuary as late as the following March. Despite this wide variation, however, a very pronounced peak in their numbers generally occurs in November. Although as previously pointed out, the February and March animals with very large guts may belong to a separate category, the typical animals with low gut and high gonadosomic ratio in these months almost certainly represent the remnants of the same group that started migrating in the late summer and autumn of the previous year.

The long period of time during which the typical forms of the River lamprey are found in the estuary is perhaps surprising in view of their marked synchrony at the time of spawning. This species for example normally reaches maturity and spawns in British rivers within a limited period of a few weeks between late March and April, the actual time depending on whether the temperature of the water has reached an apparently critical threshold values of 9-11^{o} C (Eglite, 1958a; Hagelin and Stefner, 1958; Hardisty and Potter, 1971b). It is clear from our data, however, that the animals caught in the estuary are not in exactly the same condition throughout the sampling period. For example, the gut ratio declined and the gonadosomic ratio increased between October and December. This implies that gonadal development and gut degeneration is initiated at a similar time in all individuals in the sea but that animals take a variable length of time to enter an estuarine environment on their way to fresh water and their spawning grounds.

The view that the animals in sequential monthly samples are in slightly different developmental stages is also borne out by the pattern displayed by some of the values for the body proportions. For example, between October and January the height of the second dorsal fin increased while the distance between the two dorsal fins decreased, changes known to take place before the attainment of sexual maturity (Hardisty and Potter, 1971b). Consistent patterns of change between October and December, and also often through into January, are also exhibited by the length of the prebranchial, branchial and tail region. The decline over the three months in the first of these is surprising in view of the fact that at spawning this region is longer than in early migrants. The relative increase in the prebranchial region at spawning is brought about, however, by the large decline in the length of the tail of the females and the length of the trunk of males.

That the gut degenerates rapidly in fresh water is borne out by the large differences in October between this structure in lampreys caught at Oldbury and at Tewkesbury, the latter representing animals that have only relatively recently entered fresh water. For example, the mean gut ratio for the Oldbury sample was approximately 0.8% whereas at Tewkesbury it was only 0.2%. In terms of the ovary and testis there was a less marked difference between the samples. Even here, however the gonadosomic ratio of males and females differed with values of 0.35 and 0.69 at Tewkesbury compared with 0.25 and 0.47 at Oldbury.

A conspicuous aspect of the trends exhibited by the animals caught at Oldbury was the pronounced increase in mean length of the males between October and January. This increase suggests that the

animals in each successive month may have fed both longer and in areas further from the estuary. That the pattern in the individual annual data is very much less marked and is obscured in the pooled data from different years indicates that there is a real difference in the pattern of growth between the two sexes towards the end of the marine phase. This phenomenon may be explained by the differences in the size of the gonads. For example, at the end of the marine trophic phase the females probably start diverting a larger amount of their food into the development of the gonads and relatively less into somatic growth. Such a view is consistent with both the much larger size and lipid content of the female gonad at the commencement of the spawning run (Moore and Potter, 1976b).

Although between October and January the mean lengths of males increase and those of females remain constant, the weights of the former sex during this period show little variation while those of the females exhibit a small decline. These trends are clearly reflected by the consistent drop in condition factor throughout the four months which implies that growth is becoming increasingly reduced in the population at the approach of the time of migration into the estuary. This reduction is probably related both to temperature, which is known to affect markedly the growth of lampreys, and to the increased gonadal development which heralds the onset of the upstream migration during which the animal does not feed (Hardisty and Potter, 1971b).

The question can be raised as to whether it is valid to use the term biological races for the different categories of River lampreys found in the Severn. In this context, it is pertinent to discuss to what extent interbreeding between the different groups is

likely. Much data have been published establishing that spawning in British rivers generally takes place during April (see Hardisty and Potter, 1971b). In the spawning beds on the River Teme which have been examined over many years, the population has appeared to consist exclusively of the typical form. There is also evidence that the praecox form may occur in larger numbers in smaller rivers, such as the Usk and Towy, where it has been seen spawning one to two months later than the typical form in the River Teme, a tributary of the River Severn (Hardisty, pers. comm.). A later spawning time for the praecox forms is consistent with the relatively small mean oocyte diameter in March (0.576 mm) and the fact that animals held in the laboratory did not reach maturity until late May. At the same time, the presence in March of one male with a testis containing spermatozoa suggests that this individual at least could have spawned in April at the characteristic time for the typical forms of this species.

The size difference between the two is almost certainly a function of differences in the time spent feeding in the sea. After the completion of metamorphosis, the River lamprey has been shown to migrate downstream in the spring (Potter and Huggins, 1973), although catches at Oldbury suggest that this movement may start as early as the previous autumn. From Applegate's (1950) work on the landlocked Sea lamprey it would appear that whatever time they enter the sea, substantial growth probably does not occur until the temperatures in the sea show an appreciable rise in the late spring or early summer. The estimated period for the marine trophic phase in the River lamprey is a year and a half covering the period from the spring of one year to the autumn of the next (Zanandrea, 1959; Hardisty and Potter, 1971b). It would thus seem plausible that the praecox forms represent a group

which on average had spent just under twelve months at sea migrating downstream between the autumn and spring of one year and moving back upstream in the late winter of the next.

Consistent with smaller size, the mean fecundity of the praecox forms was found in this study to be only 1,314 which is considerably lower than Hardisty's (1964) range of 7,500 - 28,000 for the typical form. Also of interest is the fact that the mean diameter of the oocyte (0.866 mm) at maturity is rather smaller than that of the typical form for which a value of approximately 1 mm has been obtained. These data support Berg's contention that the eggs of the praecox forms are smaller than those of the typical form.

While the data for the Severn Estuary certainly substantiates Berg's (1948) view that two size categories can be found in the spawning run population of River lamprey, the question of whether there are also separate summer-autumn and spring races in this species is more problematical. At the same time this concept, which has been repudiated by a number of workers (e.g. Erik, 1957; Abakumov, 1961; Eglite, 1958a, 1958b; Gaygalas and Matskevichyus, 1968), would appear to have some support from the findings of this study. Thus, the state of the guts in some of the animals of typical size caught in February and March, which are larger and better developed than in animals caught in December, suggest that a small number of animals with different characteristics commence their spawning run in the winter and early spring. That they will almost certainly spawn in the same year is borne out by the fact that their testes always contained primary spermatocytes whereas in the very early migrants from September spermatogonia could still often be observed.

There are certain aspects of the pattern displayed by the sex ratio which parallel that found in other species. Thus, pooled

data for all years showed a slight excess of males in both typical (1 ♀: 1.05 ♂) and praecox (1 ♀: 1.06 ♂) forms. In contrast, however, to the situation found in the landlocked Sea lamprey in five successive years (Potter, Beamish and Johnson, 1974) where values ranged from parity to 1 ♀: 1.15 ♂, the females were clearly predominant in 1972/73 (1 ♀: 0.84 ♂). It may be significant that there was evidence for a correlation between abundance and the proportion of males. Thus the spawning-run season when lampreys were least numerous yielded a sex ratio of 1 ♀: 0.84♂ whereas the largest excess of males (1 ♀: 1.16 ♂) was found in the year of maximum abundance. That sex ratios are related to abundance in many species has been amply demonstrated by the values obtained during the periods of increase and subsequent decline of the landlocked Sea lamprey during its invasion of the upper great lakes. Furthermore, Hardisty (1961) has shown that the large numbers of adult nonparasitic Brook lampreys, which tend to be present in alternate years, where characterised by a relatively large number of males.

River lampreys taken in estuarine conditions in the present study, show that at this stage their osmotic and ionic controlling mechanisms are unable to cope with sea water of full salinity. This contrasts sharply with the conditions observed in lampreys acclimated to 50% and 70% sea water, when there was only a transitory increase in the concentrations of the ions studied and in 50% sea water the final concentrations were similar to those previously observed by Pickering and Dockray (1972) under similar conditions.

Inverse relationship between the gonad concentrations of sodium and potassium in the livers of males and females is not readily understood but differences in lipid levels may be responsible.

The significance of high calcium levels in female gonadal tissue which increased throughout the migratory period is no doubt, in the present study, attributable to calcium-rich yolk precursors which are known to be transferred from the liver to the ovary, as established by Pickering (1976).

5. PROGESTERONE AND TESTOSTERONE

RESULTS AND DISCUSSION

In comparing the levels of progesterone and testosterone in the two species, *L. fluviatilis* and *P. marinus*, it is important to note that the terminal values are not strictly comparable from the point of view of the physiological state of the animals at the time when the samples were taken. The specimens of the Sea lamprey were animals which were actually in the process of spawning. This means that in the males the testis lobules would already have broken down to liberate the sperms into the body cavity, while in the female, the eggs would have been released from the ovarian follicles. The blood samples from River lampreys taken in May were from animals which showed secondary sex characters but which were not yet in full spawning condition.

In female River lampreys testosterone was only detected during the early migratory period in October/November (Table 16), and then in only very low concentrations (5.5 ng / 100 ml). On the other hand, relatively high concentrations of progesterone were found in the females, increasing from 31 ng / 100 ml in October/November to 1705 ng in May. At this terminal stage, progesterone concentrations were almost three times as high as in the period between February and March.

In contrast, the females of the Sea lamprey showed surprisingly high concentrations of testosterone (694 - 1669 ng / 100 ml), but progesterone levels were of a similar order to those of the female River lamprey in May.

TABLE 16

Progesterone and Testosterone concentration in the plasma of *L. fluviatilis* and sera of *P. marinus* as ng/100 ml.

SPECIES	PROGESTERONE	TESTOSTERONE	HABITAT
L. fluviatilis			
Males Oct/Nov	15.9	3331.9	Estuarine Water
Dec/Jan	-	5652.2	Estuarine Water
Feb/Mar	22.2	5126.2	Estuarine Water
May (Mature)	12.6	5344.6	Fresh Water
Females Oct/Nov	31.1	5.5	Estuarine Water
Dec/Jan	743.2	Not Detectable	Estuarine Water
Feb/Mar	638.4	Not Detectable	Estuarine Water
May (Mature)	1705.3	Not Detectable	Fresh Water
P. marinus	Range	Range	
Males (Spawning)	568.7 - 1241.4 (4)	2980 - 8846.1 (4)	Fresh Water
Females (Spawning)	1303 - 2367 (3)	694.3 - 1669.0 (3)	Fresh Water

Male River lampreys showed high levels of testosterone, increasing from 3332 ng / 100 ml in October/November to 5652 in December/January. Thereafter, there was no significant change with increasing sexual maturity. Progesterone concentrations in the males were low and showed no consistent trend throughout the migratory period. This is in contrast with conditions in the Sea lamprey males where high levels of progesterone were found, ranging between a third to three-quarters of the values recorded in the female River lampreys. On the other hand, although the highest testosterone values for male Sea lampreys exceeded the maximum value recorded for the male River lamprey, the general levels are somewhat similar in both species.

The seasonal upward trend in testosterone concentrations in male river lampreys is not inconsistent with what is known of the development of the steroidogenic tissues of the testis. As judged by its histological characteristics and the intensity of hydroxysteroid dehydrogenase activity, the interstitial tissue appears to reach its maximum development in advance of spawning and before the spermatozoa have been liberated from the testis lobules (Hardisty and Potter, 1971). The presence of comparatively high levels of testosterone in female Sea lampreys but not in River lampreys is puzzling. The explanation may lie in the difference in the sexual state of the two species used in these analyses.

However, in attempting to interpret these findings, some caution must be exercised in attributing plasma steroids exclusively to the gonadal tissues. In *vitro* studies on the interrenal tissues from feeding stages of *P. marinus* have shown that this tissue did not produce the usual range of vertebrate corticosteroids (cortisol, cortisone, corticosterone, 11-deoxycorticosterone) or testosterone,

when incubated with labelled progesterone (Weisbart and Youson, 1975). It did however, produce 11-deoxycortisol, 17α hydroxyprogesterone and androstenedione, thus demonstrating the presence of a Δ^4 biosynthesis. On the other hand, the same authors reported that incubation of testicular tissue from the same species using labelled progesterone failed to provide evidence for the formation of testosterone, although the formation of 11-deoxycorticosterone did indicate the presence of 21-hydroxylase activity. The failure to find testosterone in these experiments is almost certainly due to the relatively immature state of the testis material in the parasitic phase Sea lampreys, but the steroidogenic capacity of the interrenal raises at least the possibility that this tissue may contribute to the plasma progesterone levels and even perhaps in the females to the testosterone concentrations.

In the ovarian tissues of the cyclostome, *Eptatetrus burgeri*, evidence has been found for the existence of Δ^4 metabolic pathways leading from pregnenolone to andostenedione and for the presence of 17β-hydroxygsteroid dehydrogenase, catalyzing the conversion of androstenedione to testosterone. The presence at least in the ripe females of the Sea lamprey of high levels of testosterone may indicate the existence in the lamprey ovary of a similar system, perhaps, representing the pathway for the synthesis of oestradiol which has been reported in this species (Botticelli, 1963).

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APPENDIX 1

The mean for the length and weight and condition factor of the animal, for typical females of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	CONDITION FACTOR 10^6
November 1972	304.8 (14) ±16.52	52.2 (14) ±8.001	1.81 (14) ±1.012
December 1972	312.1 (13) ±14.041	55.0 (13) ±4.57	1.91 (13) ±0.16
January 1973	299.0 (24) ±9.66	50.1 (24) ±4.82	1.88 (24) ±0.12
February 1973	310.0 (3)	58.91 (3)	1.96 (3)

APPENDIX 2

The mean for the length and weight and condition factor of the animal, for typical males of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	CONDITION FACTOR 10^6
November 1972	311.2 (13) ±44.12	65.9 (13) ±7.43	1.89 (13) ±0.18
December 1972	314.3 (11) ±14.71	59.7 (11) ±11.031	1.88 (11) ±0.16
January 1973	316.8 (32) ±7.85	59.9 (32) ±5.62	1.89 (32) ±0.12
February 1973	322.3 (3)	63.3 (3)	1.88 (3)
March 1973	299.0 (1)	44.0 (1)	1.65 (1)

APPENDIX 3

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical females of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct 73	302.8 (12) ±23.600	57.0 (12) ±13.23	4.80 (12) ±0.59	0.69 (12) ±0.42	1.17 (12) ±0.22	1.97 (12) ±0.16
Nov 73	311.8 (30) ±11.700	58.8 (30) ±6.73	5.48 (29) ±0.74	0.37 (29) ±0.091	1.35 (29) ±0.13	1.89 (30) ±0.09
Dec 73	322.8 (8) ±18.98	58.3 (8) ±10.84	7.64 (8) ±2.96	0.42 (8) ±0.21	1.91 (8) ±0.55	1.73 (8) ±0.23
Jan 74	320.5 (6) ±26.800	56.6 (6) ±12.95	6.58 (6) ±0.67	0.64 (6) ±0.35	1.82 (6) ±0.51	1.70 (6) ±0.13
Feb 74	310.3 (3)	55.6 (3)	6.03 (3)	1.50 (3)	1.98 (3)	1.87 (3)
Mar 74	289.0 (2)	48.3 (2)	9.50 (2)	0.39 (2)	1.25 (2)	1.96 (2)
*Apr 74	267.2 (38) ±5.100	38.1 (38) ±2.38	-	0.101 (37) ±0.014	1.18 (37) ±0.11	1.98 (38) ±0.08

* Spent River lamprey caught at Tenbury.

APPENDIX 4

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical males of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct 73	285.8 (14) ±16.300	45.7 (14) ±8.69	2.40 (14) ±0.53	0.43 (14) ±0.14	1.04 (14) ±0.18	1.89 (14) ±0.12
Nov 73	302.3 (39) ±7.79	52.4 (39) ±4.32	4.42 (38) ±1.102	0.39 (38) ±0.085	1.17 (38) ±0.15	1.86 (39) ±0.072
Dec 73	309.3 (12) ±15.79	51.8 (12) ±7.45	4.41 (12) ±1.091	0.41 (12) ±0.19	1.05 (12) ±0.21	1.73 (12) ±0.14
Jan 74	354.7 (3)	70.4 (3)	-	-	-	1.58 (3)
Feb 74	29.0 (1)	41.0 (1)	6.88 (1)	0.39 (1)	1.12 (1)	1.68 (1)
Mar 74	-	-	-	-	-	-
*Apr 74	269.1 (15) ±11.700	38.2 (15) ±3.36	-	0.09 (13) ±0.02	2.06 (13) ±0.64	1.96 (15) ±0.13

* Spent River lamprey caught at Tenbury.

APPENDIX 5

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical females of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct 74	322.8 (16) ±11.600	63.9 (16) ±8.48	4.92 (12) ±0.62	0.87 (12) ±0.298	1.49 (12) ±0.19	1.88 (16) ±0.16
Nov 74	321.1 (24) ±12.19	65.0 (24) ±6.89	5.74 (19) ±0.56	0.56 (19) ±0.13	1.51(0.09) ±0.091	1.93 (24) ±0.12
Dec 74	326.7 (10) ±28.96	66.6 (10) ±13.51	5.821 (4) ±1.17	0.53 (4) ±0.19	1.54 (4) ±0.26	1.88 (10) ±0.17
Jan 75	311.2 (6) ±18.800	54.1 (6) ±8.71	8.71 (4) ±1.48	1.48 (4) ±0.23	1.59 (4) ±0.18	1.79 (6) ±0.11
Feb 75	300.3 (3)	34.4 (3)	6.28 (3)	1.44 (3)	1.94 (3)	1.31 (3)
Mar 75	285.0 (1)	37.2 (1)	7.12 (1)	0.51 (1)	1.29 (1)	
*Apr 75	259.8 (30) ±4.900	38.3 (30) ±2.64	-	0.102 (13) ±0.02	1.44 (13) ±0.13	2.18 (30) ±0.11

* Spent River lamprey caught at Tenbury.

APPENDIX 6

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical males of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct 74	291.5 (17) ±17.400	49.7 (17) ±8.801	2.58 (17) ±1.19	0.87 (17) ±0.15	1.19 (17) ±0.082	1.93 (17) ±0.14
Nov 74	309.6 (31) ±10.400	58.5 (31) ±5.41	4.45 (21) ±1.098	0.47 (21) ±0.096	1.16 (21) ±0.22	1.95 (31) ±0.102
Dec 74	312.3 (4)	60.4 (4)	4.50 (3)	0.53 (3)	1.013 (3)	1.97 (4)
Jan 75	306.0 (5) ±16.96	50.6 (6) ±8.71	4.86 (5) ±0.77	0.54 (5) ±0.26	1.21 (5) ±0.29	1.76 (6) ±0.22
Feb 75	329.0 (2)	63.21 (2)	3.58 (1)	0.199 (1)	1.34 (1),	1.72 (2)
Mar 75	-	-	-	-	-	-
*Apr 75	253.8 (21) ±8.29	36.1 (21) ±2.98	-	0.09 (7) ±0.02	1.6 (7) ±0.31	2.24 (21) ±0.26

* Spent River lamprey caught at Tenbury.

APPENDIX 7

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical females of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct 75	292.1 (12) ±13.49	52.25 (12) ±7.17	6.61 (3) ±4.22	0.60 (3) ±0.42	1.12 (3) ±0.35	2.08 (12) ±0.14
Nov 75	294.1 (38) ±9.500	50.0 (38) ±3.75	7.37 (17) ±1.12	0.37 (17) ±0.061	1.37 (17) ±0.091	1.97 (38) ±0.072
Dec 75	307.7 (41) ±7.39	54.5 (41) ±4.13	7.55 (21) ±0.88	0.37 (21) ±0.057	1.44 (21) ±0.088	1.85 (41) ±0.07
Jan 76	309.1 (14) ±12.700	53.2 (14) ±5.399	7.27 (1)	1.52 (1)	0.73 (1)	1.81 (14) ±0.17
Feb 76	304.4 (11) ±19.500	53.4 (11) ±8.061	6.60 (7) ±1.17	0.84 (7) ±0.41	1.88 (7) ±0.19	1.89 (11) ±0.202
Mar 76	290.0 (9) ±23.040	39.6 (9) ±9.44	7.62 (6) ±0.75	1.003 (6) ±1.32	2.04 (6) ±0.28	1.59 (9) ±0.101

APPENDIX 8

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical males of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits.

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct 75	272.2 (5) ±15.300	43.9 (5) ±7.54	1.96 (4) ±0.26	0.50 (4) ±0.11	1.15 (4) ±0.19	2.17 (5) ±0.17
Nov 75	287.3 (51) ±5.800	43.5 (51) ±3.081	4.09 (24) ±0.42	0.39 (24) ±0.052	1.05 (24) ±0.072	1.81 (51) ±0.061
Dec 75	301.5 (49) ±8.200	47.41 (49) ±3.54	4.83 (18) ±0.49	0.40 (18) ±0.046	1.09 (18) ±0.091	1.71 (49) ±0.072
Jan 76	301.3 (21) ±12.400	47.1 (21) ±5.39	5.56 (9) ±0.92	0.47 (9) ±0.11	1.19 (9) ±0.19	1.70 (21) ±0.11
Feb 76	277.0 (3)	38.4 (3)	4.28 (2)	1.01 (2)	1.073 (2)	1.81 (3)
Mar 76	280.8 (4)	34.6 (4)	4.48 (4)	0.81 (4)	1.40 (4)	1.56 (4)

APPENDIX 9

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical females of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits. For pooled data (1973-76).

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct.	309.6 (28) ±11.88	58.9 (28) ±5.87	5.26 (15) ±0.65	0.82 (15) ±0.16	1.44 (15) ±0.16	1.97 (28) ±0.107
Nov.	305.5 (60) ±8.14	56.0 (60) ±3.98	6.51 (36) ±0.65	0.49 (36) ±0.082	1.44 (36) ±0.065	1.94 (60) ±0.074
Dec.	311.1 (51) ±7.96	56.9 (51) ±4.21	7.27 (25) ±0.88	0.40 (25) ±0.058	1.45 (25) ±0.082	1.85 (51) ±0.060
Jan.	312.2 (26) ±8.96	54.2 (26) ±3.97	6.94 (11) ±0.57	0.67 (11) ±0.26	1.64 (11) ±0.32	1.78 (26) ±0.093
Feb.	304.7 (17) ±12.71	50.4 (17) ±6.49	6.43 (12) ±0.67	0.86 (12) ±0.32	1.91 (12) ±0.16	1.78 (17) ±0.19
Mar.	289.8 (11) ±18.62	41.3 (11) ±8.45	8.12 (8) ±1.008	0.79 (6) ±0.803	1.85 (8) ±0.43	1.66 (11) ±0.14
*Apr.	263.9 (68) ±3.64	38.2 (68) ±1.72	-	0.101 (49) ±0.012	1.24 (49) ±0.094	2.074 (68) ±0.069

* Spent River lamprey caught at Tenbury

APPENDIX 10

The mean for the length and weight and condition factor of the animal and the weight of the gonads, gut and liver (as % of the total weight) for typical males of *Lampetra fluviatilis*. Also included are the number of measurements and the ±95% confidence limits. For pooled data (1973-76).

MONTH	LENGTH	WEIGHT	GONAD	GUT	LIVER	CONDITION FACTOR 10^6
Oct.	287.1 (22) ±13.88	48.4 (22) ±6.84	2.46 (21) ±0.95	0.80 (21) ±0.14	1.18 (21) ±0.064	1.99 (22) ±0.0601
Nov.	295.7 (82) ±5.78	49.2 (82) ±3.19	4.26 (45) ±0.53	0.41 (45) ±0.052	1.10 (45) ±0.106	1.86 (82) ±0.056
Dec.	302.3 (53) ±7.66	48.4 (53) ±3.55	4.78 (21) ±0.45	0.42 (21) ±0.048	1.08 (21) ±0.085	1.73 (53) ±0.074
Jan.	307.6 (30) ±10.78	50.1 (30) ±4.75	5.31 (14) ±0.62	0.49 (14) ±0.098	1.20 (14) ±0.14	1.70 (30) ±0.086
Feb.	296.5 (6) ±33.94	4.71 (6) ±17.41	4.76 ±2.22	0.65 (4) ±0.25	1.15 (4) ±0.17	1.76 (6) ±0.203
Mar.	280.8 (4) ±34.12	34.6 (4) ±10.62	4.48 (4) ±0.89	0.81 (2) ±0.68	1.40 (4) ±0.32	1.56 (4) ±0.18
* Apr.	260.1 (36) ±7.27	37.0 (36) ±2.15	-	0.09 (20) ±0.013	1.899 (20) ±0.42	2.13 (36) ±0.16

* Spent River lamprey caught at Tenbury

APPENDIX 11

The mean ($\bar{x}$) for the body proportions (as % of total length), the number (n), and ±95% confidence limit for typical females of *Lampetra fluviatilis*. For pooled data (1973-76).

MONTH No.ANIMALS	OCT (25)	NOV (41)	DEC (35)	JAN (18)	FEB (15)	MAR (10)	APR (50)
d	5.24 ±0.19	5.35 ±0.15	5.299 ±0.108	5.20 ±0.19	5.12 ±0.29	5.04 ±0.26	5.55 ±0.13
dB_1	11.31 ±0.25	11.07 ±0.29	10.95 ±0.16	10.96 ±0.27	11.17 ±0.41	11.14 ±0.42	12.04 ±0.16
B_1B_7	10.81 ±0.22	10.55 ±0.14	10.21 ±0.18	10.60 ±0.23	10.37 ±0.34	10.38 ±0.401	11.31 ±0.22
B_7a	50.87 ±0.52	51.02 ±0.26	50.99 ±0.53	50.65 ±0.42	50.23 ±1.66	50.61 ±0.46	50.70 ±0.36
ac	27.24 ±0.49	27.43 ±0.32	27.50 ±0.33	27.97 ±0.44	27.29 ±0.59	27.74 ±0.91	25.83 ±0.39
HD_1	1.95 ±0.11	1.92 ±0.099	2.09 ±0.067	2.09 ±0.12	2.02 ±0.21	1.93 ±0.34	2.73 ±0.098
HD_2	4.37 ±0.19	4.60 ±0.15	4.76 ±0.17	4.98 ±0.23	4.67 ±0.38	4.79 ±0.36	5.71 ±0.15
nP_{pd}	3.22 ±0.35	3.20 ±0.28	3.17 ±0.32	2.95 ±0.42	3.18 ±0.47	2.72 ±0.83	0

APPENDIX 12

The mean ($\bar{x}$) for the body proportions (as % of total length), the number (n), and ±95% confidence limit for typical males of *Lampetra fluviatilis*. For pooled data (1973-76).

MONTH No.ANIMALS	OCT (22)	NOV (58)	DEC (29)	JAN (22)	FEB (6)	MAR (4)	APR (25)
d	5.31 ±0.15	5.35 ±0.11	5.33 ±0.102	5.18 ±0.11	5.11 ±0.39	5.35 ±0.39	5.86 ±0.17
dB_1	11.85 ±0.31	11.40 ±0.15	11.05 ±0.19	10.98 ±0.29	11.01 ±0.59	11.25 ±1.016	12.30 ±0.36
B_1B_7	10.67 ±0.201	10.51 ±0.11	10.35 ±0.13	10.40 ±0.25	10.37 ±0.45	9.98 ±0.21	10.87 ±0.29
B_7a	50.15 ±0.49	50.71 ±0.300	50.36 ±0.33	50.10 ±0.79	50.34 ±1.24	50.58 ±1.19	47.91 ±0.74
ac	27.57 ±0.39	27.69 ±0.26	28.36 ±0.27	28.46 ±0.92	28.33 ±1.17	28.19 ±1.28	29.07 ±0.98
HD_1	1.95 ±0.13	1.94 ±0.078	2.09 ±1.704	2.12 ±0.091	2.33 ±0.33	2.04 ±0.23	3.28 ±0.17
HD_2	4.31 ±0.19	4.60 ±0.13	4.84 ±0.13	4.99 ±0.19	5.02 ±0.29	4.71 ±0.78	6.10 ±0.19
nP_{pd}	3.33 ±0.37	3.08 ±0.202	2.92 ±0.31	2.67 ±0.27	2.97 ±0.37	2.69 ±1.19	0